3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of polybrominated biphenyls (PBBs) and polybrominated diphenyl ethers (PBDEs). It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

Polybrominated biphenyls (PBBs) and polybrominated diphenyl ethers (PBDEs) are classes of brominated hydrocarbons that are used as flame retardant additives in plastics, textiles, and other materials. Both classes of chemicals are comprised of compounds in which 1–10 bromine atoms are attached to the biphenyl structure in up to 209 different combinations. Based on the number of bromine substituents, there are 10 homologous groups of PBBs and PBDEs (monobrominated through decabrominated), each containing one or more isomers. PBBs and PBDEs are structurally similar when viewed in one dimension, differing only in the ether linkage between the two phenyl rings in PBDEs, but the oxygen bridge confers three-dimensional conformational differences that can influence toxicological properties. Consequently, on the basis of chemical structure, it cannot be assumed that the health effects of PBBs and PBDEs are necessarily similar.

Commercial production of PBBs began in approximately 1970, and manufacture was discontinued in the United States in 1976 following a contamination episode that occurred in Michigan in 1973–1974. Three main commercial mixtures of PBBs were produced: hexabromobiphenyl, octabrombiphenyl, and decabromobiphenyl. The most prevalent hexabromobiphenyl PBB mixtures had the trade names FireMaster BP-6 and FireMaster FF-1. FireMaster FF-1 was produced by grinding FireMaster BP-6 and adding 2% calcium polysilicate as an anticaking agent. The hexabromobiphenyl mixtures contained varying proportions (depending on lot number) of di- through octabrominated homologues, and 2,2',4,4',5,5'-hexabromobiphenyl was the most abundant congener (53.9–68.0%) followed by 2,2',3,4,4',5,5'-heptabromobiphenyl (7.0–27.3%). Commercial octabromobiphenyl PBB mixtures contained a large proportion (47.4–60.0%) of nonabromobiphenyl congeners, whereas commercial

decabromobiphenyls contained predominately (96.8%) decabromobiphenyl congener. The general names hexabromobiphenyl, octabromobiphenyl, and decabromobiphenyl are used in this profile to refer to unspecified commercial mixtures of these PBBs.

Concern regarding the health effects of PBBs is largely related to exposures that resulted from the Michigan contamination episode. Livestock on farms in Michigan were exposed to FireMaster FF-1 over a period of approximately 10 months after it was accidentally mistaken for the feed supplement magnesium oxide and mixed with animal feed that was distributed within the state. Health problems in dairy cattle (decreased feed consumption and decreased milk production), reported in the fall of 1973, were the first signs that the contamination episode occurred, but accidental addition of PBBs to animal feed was not identified as the cause of the problem until late spring of 1974 (Fries 1985a; Jackson and Halbert 1974). The U.S. Food and Drug Administration (FDA) established tolerances of 1 ppm in milk and meat fat and 0.1 ppm in eggs in May 1974, which were revised downward to 0.3 and 0.05 ppm, respectively, in November 1974 due to improved analytical sensitivity (Dunckel 1975; Fries 1985a). The Michigan Department of Agriculture (MDA) subsequently lowered the FDA tolerance in meat fat from 0.3 to 0.02 ppm, but there currently are no FDA or MDA tolerances for PBBs (FDA 1989; Fries 1985a). As a result of a farm animal testing and quarantining program established by the MDA in May 1974, about 30,000 dairy cattle, 2,000 swine, 400 sheep, and over 2,000,000 chickens were found to contain PBBs at concentrations requiring their destruction (Dunckel 1975; Fries 1985a; Mercer et al. 1976).

Most of the information that is available on health effects of PBBs in humans comes from studies of Michigan residents who ingested milk, meat, and eggs that were produced on farms that used the FireMaster-contaminated animal feed. In the interval of more than 9 months between the accident, the detection and identification of its cause, and the beginning of testing and the establishment of quarantines, PBB-contaminated food products were consumed, not only by farm families and people that acquired produce directly from PBB-contaminated farms, but also by people who purchased food from markets (Anderson et al. 1979). The Michigan PBB contamination episode led to the establishment of epidemiological studies (that are still ongoing) of Michigan residents who were expected to have consumed PBB-contaminated food, as well as to a substantial increase in research activity regarding the health effects of PBBs in cattle, poultry, and laboratory animals. Compared to the FireMaster (commercial hexabromobiphenyl) PBBs, relatively limited data are available on health effects of commercial mixtures of octabromobiphenyl and decabromobiphenyl. Reviews of the research results on the toxicity of PBBs include those by Damstra et al. (1982), DiCarlo et al. (1978), Fries (1985a), Kay (1977), Kimbrough (1987), Kimbrough et al. (1978a), and WHO (1994a).

This profile discusses information regarding health effects of PBBs in humans and laboratory animals; some research on cattle and poultry is also discussed, but its relevance to human health effects is uncertain due to interspecies physiological differences. Although livestock toxicity at high-level farms is generally concluded to have been due to PBBs, data regarding effects at farms with low levels of PBBs have generated some controversy because signs of toxicosis reported in Michigan cattle have not been reproduced in animals exposed to PBBs in controlled experiments that led to residue concentrations in tissues 100 times greater than those reported in the Michigan animals (Jackson and Halbert 1974; Moorhead et al. 1977). This led some investigators to suggest that some signs of toxicosis reported in Michigan cattle reflected farm management procedures, nutritional deficiencies, microbial and parasitic infections, or exposure to unknown contaminants in the feed (Durst et al. 1977; Fries 1985a; Moorhead et al. 1977). Although exposure by ingestion occurred during the Michigan contamination episode, existing information on the metabolism of PBBs in livestock is insufficient to ascertain whether the people ingested PBBs or metabolic products of PBBs. However, based on available data discussed in Section 3.4.2.2, it is reasonable to assume that mainly unchanged penta-, hexa-, and heptabromobiphenyl congeners in animal products were consumed.

Unlike PBBs, PBDEs have been continuously produced and used as flame-retardant additives since the 1970s. Concern for health effects of PBDEs has heightened due to relatively recent evidence that they have become ubiquitously distributed in the environment and are present in tissues and breast milk of the general population at levels that continue to increase. Three commercial PBDE mixtures have been and continue to be produced: decabromodiphenyl ether (decaBDE), octabromodiphenyl ether (octaBDE), and pentabromodiphenyl ether (pentaBDE). DecaBDE has accounted for more than 80% of PBDE usage. The composition of commercial decaBDE is $97% of the pure congener with the remainder mainly nonaBDE. Commercial octaBDE is a mixture of congeners ranging from nona- to hexaBDE, and mixtures of pentaBDE are comprised of tetra-, penta-, and hexaBDE congeners. Congeners with less than four bromine atoms are generally not found in commercial PBDEs. Reviews on the health effects and other aspects of PBDEs include those by Darnerud et al. (2001), de Boer et al. 2000, de Wit (2002), Hardy (1999, 2002a, 2002b), McDonald (2002), NAS (2002), Rahman et al. (2001), Silberhorn et al. (1990), and WHO (1994b).

Toxicity data for individual PBB and PBDE congeners are included in some discussions in this profile when these data corroborate or provide information on effects not documented for the PBB and PBDE mixtures. Congener-specific toxicity data are currently not practical for determining exposure levels of PBB and PBDE mixtures associated with adverse health effects at hazardous waste sites. This is due in

part to the fact that standardized analytical procedures for congener mixtures and commercially available standards for all congeners are lacking, and congener-specific analyses are not routinely performed. Additionally, using current health effects evaluation procedures, toxicity data for individual congeners may overestimate or underestimate the actual health risk of PBB and PBDE mixtures because congeners vary in toxic potency and may be influenced by other congeners in an additive or less-than-additive way. It is also important to recognize that the PBBs and PBDEs to which people may be exposed are likely to be different from the original PBB and PBDE source because of changes in congener composition resulting from differential partitioning and transformation in the environment and differential biological metabolism and retention.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not

the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of PBBs and PBDEs are indicated in Tables 3-1, 3-2 and 3-3 and Figures 3-1 and 3-2. Because cancer effects could occur at lower exposure levels, Figure 3-1 also shows a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for PBBs and PBDEs. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

3.2.1 Inhalation Exposure

A few studies have examined groups of chemical workers involved in the manufacture and distribution of PBBs and/or PBDEs (Bahn et al. 1980; Brown et al. 1981; Chanda et al. 1982; Landrigan et al. 1979; Rosenman et al. 1979; Stross et al. 1981). These people are believed to have been exposed predominately by dermal contact and inhalation, although the oral route cannot be ruled out. Results from these studies, therefore, are discussed in this section as well as in Section 3.2.3.

3.2.1.1 Death

Polybrominated Biphenyls. No studies were located regarding death in humans after inhalation exposure to PBBs.

Nose-only exposure to the highest attainable dust concentration of octabromobiphenyl mixture for 4 hours (960 mg/m^3 as a time-weighted average) was not lethal to six male rats observed for 7 days (Waritz et al. 1977). No deaths occurred in groups of five male and five female rats that were exposed to a decabromobiphenyl dust mixture at concentrations ranging from 5 to 5,000 mg/m^3 concurrently with starch dust (6 mg/m^3) for 6 hours/day, 5 days/week for 4 weeks (Millischer et al. 1980). Information on lethality of inhaled hexabromobiphenyl PBB mixtures was not located.

Polybrominated Diphenyl Ethers. No studies were located regarding death in humans after inhalation exposure to PBDEs.

No deaths occurred in groups of five male and five female rats that were chamber-exposed to pentaBDE aerosol (compound dissolved in corn oil), octaBDE dust, or decaBDE dust in concentrations as high as 200,000, 60,000, or 48,200 mg/m^3, respectively, for 1 hour and observed for the following 14 days (IRDC 1974, 1975a, 1975b). Confidence in these studies is limited by a lack of control data. There was no mortality in rats that were exposed to #174 mg/m^3 of octaBDE dust for 8 hours/day for 14 consecutive days (IRDC 1978).

3.2.1.2 Systemic Effects

No studies were located regarding cardiovascular, gastrointestinal, or musculoskeletal effects in humans or animals after inhalation exposure to PBBs.

No studies were located regarding musculoskeletal or dermal effects in humans or animals after inhalation exposure to PBDEs.

Systemic effects that have been observed in humans and animals following inhalation exposure to PBBs and PBDEs are described below.

Respiratory Effects.

Polybrominated Biphenyls. No studies were located regarding respiratory effects in humans after inhalation exposure to PBBs.

Slight dyspnea was observed in five male and five female rats that were exposed to a decabromobiphenyl dust mixture at 5,000 mg/m^3 concurrently with starch dust (6 mg/m^3) for 6 hours/day, 5 days/week for 4 weeks (Millischer et al. 1980). This effect was not observed at 500 mg/m^3 and lower concentrations or in air only-exposed controls, and there were no changes in pulmonary resistance and compliance in urethane-anesthetized rats, blood gases, and lung histology at any of the exposure levels. Lung function and blood gases were not evaluated in starch-exposed controls, but this is unlikely to be a serious study deficiency as the ratio of PBB to starch was . 1,000 in the high exposure group.

Polybrominated Diphenyl Ethers. No studies were located regarding respiratory effects in humans after inhalation exposure to PBDEs.

Transient signs of respiratory distress that included tachynpea or dyspnea developed in rats that were chamber-exposed to pentaBDE aerosol (compound dissolved in corn oil), octaBDE dust, or decaBDE dust in very high concentrations of 200,000, 60,000, and 48,200 mg/m^3, respectively, for 1 hour (IRDC 1974, 1975a, 1975b). Confidence in these effect levels is low due to a small number of tested animals and lack of control data. Increased respiration rate also occurred in rats that were exposed to $24.4 mg/m^3 concentrations of octaBDE dust for 8 hours/day for 14 consecutive days (IRDC 1978). The effect was observed by the end of each exposure period, always disappeared by the following morning,

and was not observed at lower concentrations of #3.68 mg/m^3. Histological examinations of rats exposed to 174 mg/m^3 in the 14-day study showed no effects in the nasal turbinates, trachea, or lungs.

Cardiovascular Effects.

Polybrominated Diphenyl Ethers. No studies were located regarding cardiovascular effects in humans after inhalation exposure to PBDEs.

No histopathological changes were observed in the hearts of rats that were exposed to 174 mg/m^3 of octaBDE dust for 8 hours/day for 14 consecutive days (IRDC 1978).

Gastrointestinal Effects.

Polybrominated Diphenyl Ethers. No studies were located regarding gastrointestinal effects in humans after inhalation exposure to PBDEs.

No histopathological changes were observed in the stomach, duodenum, or colon of rats that were exposed to 174 mg/m^3 of octaBDE dust for 8 hours/day for 14 consecutive days (IRDC 1978).

Hematological Effects.

Polybrominated Biphenyls. No studies were located regarding hematological effects in humans after inhalation exposure to PBBs.

Hematology was normal in groups of five male and five female rats that were exposed to a decabromobiphenyl dust mixture at concentrations of 5 or 5,000 mg/m^3 concurrently with starch dust (6 mg/m^3) for 6 hours/day, 5 days/week for 4 weeks (Millischer et al. 1980). The evaluation included erythrocyte and leucocyte counts, differential leukocyte count, hematocrit, and hemoglobin level.

Polybrominated Diphenyl Ethers. No studies were located regarding hematological effects in humans after inhalation exposure to PBDEs.

No adverse hematological changes were observed in rats that were exposed to 24.4 or 174 mg/m^3 of octaBDE dust for 8 hours/day for 14 consecutive days (IRDC 1978). Evaluation of hemoglobin,

hematocrit, total erythrocyte count, and total and differential leukocyte counts showed no remarkable responses except for an elevation in leukocyte numbers. The observed increase in leukocyte counts was considered to be an unusual response by the investigators, although it was within the normal range for control rats in their laboratory.

Hepatic Effects.

Polybrominated Biphenyls. No studies were located regarding hepatic effects in humans after inhalation exposure to PBBs.

No significant ($p<0.05$) increase in relative liver weight or hepatic histological changes were found in six male rats nose-only exposed to a octabromobiphenyl dust mixture at 960 mg/m^3 for 4 hours (time-weighted average, highest attainable concentration), and observed for 7 days (Waritz et al. 1977). Toxicity of octabromobiphenyl mixture vapor was investigated in groups of six rats almost continuously exposed (23 hours/day, 7 days/week) for 2, 4, 7, 9, 11, 13, or 15 weeks (Waritz et al. 1977). The exposure level was 0.00035 µg/m^3, which is the reported equilibrium concentration at 28 EC. Gross pathologic examination and measurement of relative liver weight showed no exposure-related changes at any of the sacrifices, but it is unclear if liver histology was evaluated.

Relative liver weight was increased . 25% in groups of 5 or 10 rats that were exposed to a decabromobiphenyl dust mixture at concentrations of 50–5,000 mg/m^3 concurrently with starch dust (6 mg/m^3) for 6 hours/day, 5 days/week for 4 weeks (Millischer et al. 1980). The increased liver weight was not accompanied by hepatic histologic changes, and therefore may be an adaptive response because PBBs are hepatic inducers and cause cellular proliferation (see Section 3.2.2.2 Hepatic Effects). No effects on liver weight or histology were observed at 5 mg/m^3.

Polybrominated Diphenyl Ethers. No studies were located regarding hepatic effects in humans after inhalation exposure to PBDEs.

Increased liver weight and hepatic histological changes were observed in rats that were exposed to $3.6 mg/m^3 concentrations of octaBDE dust for 8 hours/day for 14 consecutive days (IRDC 1978). At 3.6 mg/m^3, the liver lesions consisted of very slight to slight, focal to multifocal cytoplasmic enlargement of the hepatocytes, accompanied by focal acidophilic degeneration of individual to small groups of cells. The liver lesions were similar at higher concentrations (24.4 or 174 mg/m^3), except that the hepatocyte

enlargement was multifocal to diffuse in distribution and was accompanied by focal, small to large areas of hepatocellular necrosis of very slight to marked degree. No liver effects were found at 0.58 mg/m^3 (lowest tested concentration).

Renal Effects.

Polybrominated Biphenyls. No studies were located regarding renal effects in humans after inhalation exposure to PBBs.

Groups of six rats were exposed to 0.00035 µg/m^3 of octabromobiphenyl mixture vapor (equilibrium concentration) 23 hours/day, 7 days/week for 2, 4, 7, 9, 11, 13, or 15 weeks (Waritz et al. 1977). Gross pathologic examination at each sacrifice and measurement of relative kidney weight at the last sacrifice showed no exposure-related changes, but it is unclear if kidney histology was evaluated.

Urinalysis was normal in groups of five male and five female rats that were exposed to a decabromobiphenyl dust mixture at concentrations ranging from 5 to 5,000 mg/m^3 concurrently with starch dust (6 mg/m^3) for 6 hours/day, 5 days/week for 4 weeks (Millischer et al. 1980). The analysis included pH, specific gravity, proteins, glucose, ketone bodies, biliary pigments, urobilinogen, blood, and microscopic examination of sediment. A comprehensive histology evaluation was performed in this study, but the only tissues specifically mentioned as having been examined are the liver and lungs. However, a total of 21 tissues were examined; therefore, it is probable that the kidney was examined, but was not discussed because no histological alterations were found.

Polybrominated Diphenyl Ethers. No studies were located renal respiratory effects in humans after inhalation exposure to PBDEs.

No histopathological changes were observed in the kidneys or urinary bladder of rats that were exposed to 174 mg/m^3 of octaBDE dust for 8 hours/day for 14 consecutive days (IRDC 1978).

Endocrine Effects.

Polybrominated Biphenyls. Hypothyroidism was diagnosed in 4 of 35 men who were occupationally exposed to unspecified PBBs and/or decaBDE (Bahn et al. 1980). The cohort consisted of workers (mean age 35.9 years) who had been employed at a production plant for at least 6 weeks during a 52-month

period during which PBBs and decaBDE were the only chemicals manufactured and who had volunteered for a comprehensive medical evaluation performed 3 months after the end of the 52-month period. There was no further description of exposure, and it was assumed to have occurred by both inhalation and dermal routes. The cohort was matched by sex, race, and age to 89 unexposed control subjects. Four subjects (22–50 years old, employed for 9–46 months not entirely during the 52-month production period) had elevated serum thyrotropin levels (mean 37.5 versus #1.5–8 µU/ml normal range), low or borderline low serum thyroxine (T_4) levels (4.4 versus 4.5–11.5 µg/dL) and free-thyroxine indices (3.7 versus 3.8–10.8), and markedly elevated thyroid antimicrosomal antibody titers (1:6,400 or above). Serum T_4 levels measured 7 months earlier in two of the four men were normal. Antithyroglobulin antibodies were elevated in one of the four subjects (not evaluated in other workers). The exposed cohort had significantly more subjects with elevated serum thyrotropin ($p=0.006$), but free thyroxine index ($p=0.06$), serum T_4 level ($p=0.11$) and antimicrosomal antibody titer ($p=0.06$) did not differ significantly from the controls. Questioning about exposure to 74 occupational toxicants showed that three of the four hypothyroid subjects had only three common chemical exposures (PBBs, decaBDE, and bromine); the fourth worker was hired after PBB production ceased and was exposed only to decaBDE and bromine, but it is not clear if PBBs were still present in the work environment. Except for one control subject who had an enlarged thyroid, none of the exposed or control subjects had signs of thyroid enlargement, thyroid nodularity or hypothyroidism on physical examination, or had reported taking thyroid medication or having thyroid problems within the previous 5 years. Reevaluation of three of the four subjects 1 year later (none had been treated with thyroid hormone) showed that two still had low free-thyroxine indices and high serum thyrotropin, one had a normal free-thyroxine index and a high-normal serum thyrotropin, and all three still had markedly elevated thyroid antimicrosomal antibody titers. The findings of this study suggest that occupational exposure to PBBs, decaBDE, and/or bromine affected the thyroid, but the mixed chemical exposure and a lack of data on serum or tissue levels of the chemicals preclude attributing effects solely to any particular congener or mixture of congeners.

Polybrominated Diphenyl Ethers. There is suggestive evidence of hypothyroidism in a small group of workers who were occupationally exposed to decaBDE as well as PBBs (Bahn et al. 1980), as summarized in the preceding subsection on endocrine effects of PBBs.

No histopathological changes were observed in the thyroid, parathyroid, adrenal, or pituitary of rats that were exposed to 174 mg/m^3 of octaBDE dust for 8 hours/day for 14 consecutive days (IRDC 1978).

Dermal Effects.

Polybrominated Biphenyls. In a medical history survey study, 7 of 10 (70%) workers in the production department of a PBB manufacturing plant reported that they experienced symptoms of skin disorders, compared with 31% of 45 workers in other departments in the same plant and 18% in a control group of 153 Wisconsin farm residents (Chanda et al. 1982). The survey covered a period of 3 years of potential exposure, but exposure levels were not reported. The dermatological symptoms were described as "almost uniformly" halogen acne (bromacne). Mean serum PBB levels for the respective PBB groups (with ranges listed in parentheses) were 603.9 ppb (11.4–1,729 ppb) and 16.5 ppb (4–234 ppb); PBBs were not detected in serum of the control subjects (Chanda et al. 1982). Physical examination confirmed the occurrence of bromacne in 13% of PBB workers compared with no acne in the control group. No other studies were located regarding dermal effects in humans after occupational exposure to PBBs.

No studies were located regarding dermal effects in animals after inhalation exposure to PBBs.

Ocular Effects.

Polybrominated Biphenyls. No studies were located regarding ocular effects in humans after inhalation exposure to PBBs.

Signs of ocular irritation (no further description) were observed in five male and five female rats that were exposed to a decabromobiphenyl dust mixture at 5,000 mg/m^3 concurrently with starch dust (6 mg/m^3) for 6 hours/day, 5 days/week for 4 weeks (Millischer et al. 1980). The seriousness of this effect is unclear as severity was not reported and recovery was not assessed. Ocular irritation was not observed at 500 mg/m^3 and lower concentrations.

Polybrominated Diphenyl Ethers. No studies were located regarding ocular effects in humans after inhalation exposure to PBDEs.

Transient signs of ocular irritation that included eye squint, erythema, and/or ocular discharge were observed in rats that were chamber-exposed to pentaBDE aerosol (compound dissolved in corn oil), octaBDE dust, or decaBDE dust in concentrations of 2,000, 2,000, and 48,200 mg/m^3, respectively, for 1 hour (IRDC 1974, 1975a, 1975b). Confidence in these effect levels is low due to a small number of

tested animals and lack of control data. No histopathological changes were observed in eyes of rats that were exposed to 174 mg/m^3 of octaBDE dust for 8 hours/day for 14 consecutive days (IRDC 1978).

3.2.1.3 Immunological and Lymphoreticular Effects

Polybrominated Biphenyls. Several immunological parameters were evaluated in a group of 28 workers from the Michigan Chemical Company who were involved in manufacturing and distributing PBBs (Stross et al. 1981). This company manufactured the FireMaster FF-1 that was involved in the agricultural contamination episode in Michigan in 1973–1974. The subjects had worked directly with PBBs during the previous 5 years, but exposure levels were not reported. Immunological analyses included determination of immunoglobulin levels, skin testing, and lymphocyte transformation studies. No abnormalities in lymphocyte number or function could be determined when compared to an unexposed group. One of three blastogenic responses (pokeweed mitogen [PWM]) was significantly reduced ($p<0.01$) relative to concurrent controls, but was within the normal control range for the laboratory. PWM is a mitogenic lectin that stimulates both human T and B cells. No specific information was provided regarding the skin testing and immunoglobulin levels.

No studies were located regarding immunological or lymphoreticular effects in animals after inhalation exposure to PBBs.

Polybrominated Diphenyl Ethers. No studies were located regarding immunological or lymphoreticular effects in humans after inhalation exposure to PBDEs.

No histopathological changes were observed in lymph nodes or bone marrow from rats that were exposed to 174 mg/m^3 of octaBDE dust for 8 hours/day for 14 consecutive days (IRDC 1978).

3.2.1.4 Neurological Effects

Polybrominated Biphenyls. Twenty-five workers at a PBB-manufacturing plant (exposure duration and levels not reported) displayed mean scores on tests of memory and learning that were typical for people of their age, and educational, occupational, and cultural backgrounds, even though they had an elevated mean PBB concentration in adipose tissue (9.33 ppm) (Brown et al. 1981). Workers with the highest concentrations of PBBs in adipose tissue showed no evidence of memory dysfunction in these tests.

No studies were located regarding neurological effects in animals after inhalation exposure to PBBs.

Polybrominated Diphenyl Ethers. No studies were located regarding neurological effects in humans or animals after inhalation exposure to PBDEs.

3.2.1.5 Reproductive Effects

Polybrominated Biphenyls. Eleven workers in a PBB manufacturing company (exposure duration and levels not reported) displayed no differences in the distribution of sperm counts, motility, or sperm morphology compared with a control group of 52 nonexposed men (Rosenman et al. 1979). PBBs were detected in the serum of all exposed subjects and in only one nonexposed subject, but no mean or individual serum PBB values were reported.

No studies were located regarding reproductive effects in animals after inhalation exposure to PBBs.

Polybrominated Diphenyl Ethers. No studies were located regarding reproductive effects in humans after inhalation exposure to PBDEs.

No histopathological changes were observed in ovaries or testes from rats that were exposed to 174 mg/m^3 of octaBDE dust for 8 hours/day for 14 consecutive days (IRDC 1978).

3.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after inhalation exposure to PBBs or PBDEs.

3.2.1.7 Cancer

No studies were located regarding cancer in humans or animals after inhalation exposure to PBBs or PBDEs.

3.2.2 Oral Exposure

No studies were located regarding cancer in humans or animals after inhalation exposure to PBBs.

3.2.2.1 Death

Polybrominated Biphenyls. No studies were located regarding death in humans after oral exposure to PBBs.

Limited information is available on lethal amounts of PBBs in animals. In general, dosing regimen and magnitude affect response. The lack of decreased survival in some studies does not necessarily indicate low toxicity because observation periods may not be sufficient to observe effects that develop slowly.

Except as noted below, acute-duration studies administered PBBs by gavage in oil vehicle. A single 1,000 mg/kg dose of FireMaster FF-1 did not significantly increase mortality in rats observed for #2 years posttreatment (Kimbrough et al. 1978b, 1981). Exposing pregnant rats to #800 mg/kg FireMaster BP-6 on one of gestation days 6–14 did not significantly increase mortality, but the animals were not observed beyond pregnancy (Beaudoin 1977). Administration of 1,000 mg/kg/day FireMaster FF-1 for 6–10 doses (5 days/week), however, caused 100% mortality in rats; the mean time to death was 12.3 days in females and 11.0 days in males (Gupta and Moore 1979). The cause of death was not specifically reported, but a general statement indicated that the rats had hunchback posture, rough coat, and sunken eyes, were lethargic, and appeared dehydrated and emaciated. No deaths occurred in rats administered octabromobiphenyl mixture in a single dose #1,000 mg/kg with 4 weeks of observation (Lee et al. 1975a), 2,000 mg/kg with 2 weeks of observation (Norris et al. 1975a), 17,000 mg/kg with 1 week of

observation (Lee et al. 1975a; Waritz et al. 1977), or 3,000 mg/kg/day on 2 consecutive days with 4 weeks of observation (Lee et al. 1975a). The 17,000 mg/kg dose was the highest that was feasible to administer, apparently due to gavage volume because it had to be administered as divided doses given in a 4-hour period. Dietary administration of octabromobiphenyl mixture in estimated dosages of #70 mg/kg/day for 2 weeks was not lethal in rats, but there was no posttreatment observation period (Lee et al. 1975b; Waritz et al. 1977). In the only study of a decabromobiphenyl mixture, a single dose as high as 5,000 mg/kg caused no deaths in rats observed for 14 days (Millischer et al. 1980). In mice, dietary administration of FireMaster BP-6 for 2 weeks produced death (cause not reported) at estimated doses of 130 mg/kg/day, but not #36 mg/kg/day (Cagen et al. 1977; Fraker 1980; Fraker and Aust 1980). Information on acute oral lethality in species other than rats and mice was not located.

In intermediate-duration studies with rats, no deaths were induced by dietary administration of FireMaster BP-6 at estimated dosages of #5 mg/kg/day for #82 days (Darjono et al. 1983) or #10 mg/kg/day for 30 days (Akoso et al. 1982a). No deaths were observed in rats fed #50 mg/kg/day of an unspecified PBB mixture for 30 or 60 days (Sleight and Sanger 1976). Twice weekly gavage with 100 mg/kg FireMaster FF-1 in corn oil for two 3-week dosing periods, separated by . 6 weeks, was not lethal in rats observed for 2 years (Kimbrough et al. 1981). Twenty-two gavage doses of 100 mg/kg FireMaster FF-1 in corn oil (5 days/week for 4.5 weeks) produced 38 and 100% mortality in male and female rats, respectively; the average times to death were 46.7 and 60.3 days, respectively (Gupta and Moore 1979). Similar treatment with 30 mg/kg/day FireMaster FF-1 was not lethal in rats observed for . 5 months. Based on these gavage data, the calculated LD_{50} in rats observed for . 60 days posttreatment (i.e., 90-day $LD_{50)}$ was 149 and 65 mg/kg/day for male and female rats, respectively (Gupta and Moore 1979). This study did not specifically address the cause of death, but emaciated appearance and gross loss of subcutaneous and visceral adipose tissue indicate wasting was a contributing factor. Rats that were treated with FireMaster FF-1 in corn oil by gavage on 5 days/week for 25 weeks exhibited dose-related decreased survival at $0.3 mg/kg/day (cause of death not discussed), but not at 0.1 mg/kg/day (NTP 1983). The decreased survival was only apparent when the rats were observed for a lifetime (. 15–22 months posttreatment) and consistent only in males. Survival was also decreased in male but not female rats given $0.5 mg/kg/day FireMaster FF-1 in the diet for up to 104 weeks (Chhabra et al. 1993; NTP 1992). The decreased survival appeared to be related to increased incidences of mononuclear cell leukemia. No deaths were observed in rats treated with octabromobiphenyl mixture in the diet at estimated dosages of #71 mg/kg/day for 4 weeks and observed for #18 weeks (Lee et al. 1975b; Waritz et al. 1977). Rats treated with #1 mg/kg/day dietary octabromobiphenyl mixture for 8 months did not die, but there were some deaths (number and cause not reported) in rats treated with higher dietary dosages (8–800 mg/kg/day) for

30 days (Norris et al. 1975a). Insufficient information is available to determine if the deaths were treatment-related, since incidences and other pertinent information were not reported.

Survival data for intermediate-duration exposure to PBBs are less extensive for species other than rat, but indicate that guinea pigs and mink are particularly susceptible. High mortality occurred in guinea pigs fed estimated dosages of 2 mg/kg/day FireMaster BP-6 for 45 days (Vos and van Genderen 1973, 1974) or $4 mg/kg/day of an unspecified PBB mixture for 30 days (Sleight and Sanger 1976); dosages of #0.4 mg/kg/day of either mixture were not lethal. The Litchfield and Wilcoxon procedure was used to calculate dietary LD_{50} values of 0.47 and 0.61 mg/kg/day (estimated dosages) for male and female mink, respectively, exposed to FireMaster FF-1 for life (63–294 days) (Aulerich and Ringer 1979; Ringer et al. 1981). Dosages #0.18 mg/kg/day did not significantly increase mortality in the mink. Dietary administration of FireMaster BP-6 in an estimated dosage of 21.7 mg/kg/day for 12 weeks caused some deaths in mice (number not reported), leading to sacrifice of other test animals (Martino et al. 1981; Wilson-Martino et al. 1980). Mean survival time decreased significantly in female mice treated with 10 mg/kg/day of FireMaster FF-1 in corn oil by gavage on 5 days/week for 25 weeks, but not #3 mg/kg/day (NTP 1983). Decreased survival was only apparent when the mice were observed for #24 months posttreatment (lifetime observation) and not observed in similarly treated males. Survival was also decreased in female mice given $1.3 mg/kg/day FireMaster FF-1 in the diet for up to 105 weeks; decreased survival occurred in similarly treated male mice at 3.9 mg/kg/day (Chhabra et al. 1993; NTP 1992). The cause of death was not discussed in the NTP(1983, 1992) mouse studies, but hepatocellular tumors increased significantly in both sexes at dosages that decreased survival.

No deaths occurred in two swine that ingested estimated dosages of #8 mg/kg/day for 16 weeks; one pig was observed for 102 days following exposure and the other for 14 weeks following exposure (Ku et al. 1978). An adult male monkey died after consuming 0.73 mg/kg/day FireMaster FF-1 in the diet for 25 weeks (Allen et al. 1978; Lambrecht et al. 1978). The death was attributed to severe gastrointestinal changes, including ulcerative colitis. The only other animal in this study was a juvenile female who survived 50 weeks of a dietary dosage of 1.43 mg/kg/day. In another study, one juvenile female monkey that consumed 18 mg/kg/day FireMaster FF-1 in the diet died after 137 days of continuous exposure (Allen et al. 1978). Although only one or two monkeys were tested in these studies, effects characteristic of PBB poisoning (e.g., dermal changes, body weight loss) indicate that the deaths were exposure-related. Pregnant cows given 67 mg/kg/day FireMaster BP-6 in capsules for 60 days (dosing began $10 days after pregnancy diagnosis) were sacrificed between days 33 and 66 because of impending death (Moorhead et al. 1977). Clinical signs developed progressively and included anorexia, emaciation, and depressed

general condition. No mortality occurred in cows treated with #0.65 mg/kg/day and observed for 1 or 140 days following the end of treatment.

The LD_{50} value and reliable LOAEL values for death in each species in the acute- and intermediate-duration categories are recorded in Table 3-1 and plotted in Figure 3-1.

Polybrominated Diphenyl Ethers. No deaths occurred in rats that were treated with a single gavage dose of #5,000 mg/kg of decaBDE or #2,000 mg/kg of 77.4% decaBDE (containing 21.8% nonaBDE and 0.8% octaDBE) and observed for the following 14 days (IRDC 1974; Norris et al. 1975b). No mortality was observed in rats and mice that were exposed to decaBDE via diet in estimated doses of #16,000 and #19,000 mg/kg/day, respectively, for 14 days (NTP 1986).

In intermediate-duration dietary studies with decaBDE, there was no exposure-related mortality in rats that were exposed to estimated dietary doses of #90 mg/kg/day for 28 days (IRDC 1976) or rats and mice fed estimated doses of #8,000 and #9,500 mg/kg/day, respectively, for 13 weeks (NTP 1986). In chronic studies, there were no effects on survival in rats that were fed 0.01–1.0 mg/kg/day of a 77.4% decaBDE mixture (containing 21.8% nonaBDE and 0.8% octaDBE) for 2 years (Kociba et al. 1975; Norris et al. 1975b), or in rats and mice fed decaBDE in estimated doses of #2,550 and #7,780 mg/kg/day, respectively, for 103 weeks (NTP 1986).

No deaths occurred in rats that were administered octaBDE by gavage in single doses #5,000 mg/kg and observed for the following 14 days (IRDC 1975a). Intermediate-duration dietary studies with octaBDE, observed no mortality in rats exposed to estimated dietary doses of #90 mg/kg/day for 28 days or #750 mg/kg/day for 13 weeks (IRDC 1976, 1977).

Single-dose gavage LD_{50} values of 5,000 and 6,200 mg/kg were determined for pentaBDE (Saytex 115 and DE-71, respectively) in rats that were observed for 14 days (British Industrial Biological Research Association 1977; Pharmakon Research International Inc. 1984). Another study found that a single 5,000 mg/kg dose of pentaBDE caused deaths in four of five rats in the 14 days following treatment,

Table 3-1. Levels of Significant Exposure to PBBs - Oral

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	ACUTE EXPOSURE						
	Death						
1	Rat (Fischer 344/N)	2 wk 5d/wk 1x/d (GO)				1000 (18/18 died)	Gupta and Moore 1979 (FF-1)
2	Mouse (Balb/c)	14 d ad lib (F)				130 F (63% lethality)	Fraker 1980 Fraker and Aust 1978 (BP-6)
	Systemic						
3	Rat (Sprague-Dawley)	10 d 1x/d (GO)	Endocr	1[b]	3 M (decreased thyroid plasma T4 hormone)		Allen-Rowland s et al. 1981 (NS)
4	Rat (Sprague-Dawley)	once (GO)	Endocr	286 M			Allen-Rowland s et al. 1981 (NS)
5	Rat (Wistar)	once Gd 6-14 (GO)	Bd Wt	400 F	800 F (unknown percent maternal weight loss)		Beaudoin 1977 (BP-6)
6	Rat (Sherman)	once 18 mo observa-tion (GO)	Hepatic		500 M (increased hepatic phospholipids and serum cholesterol)		Bernert et al. 1983 (FF-1)

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
7	Rat (Fischer 344/N)	2 wk 5 d/wk 1x/d (GO)	Hepatic		1000	(hepatocytic swelling, fatty infiltration, multinucleation, necrosis, and cytolysis)			Gupta and Moore 1979 (FF-1)
			Renal		1000	(darkened kidneys)			
			Endocr		1000	(darkened adrenal glands)			
			Bd Wt				1000	(unknown percent weight loss, emaciation)	
8	Rat (Fischer 344/N)	2 wk 5 d/wk 1x/d (GO)	Resp	30					Gupta et al. 1981 (FF-1)
			Cardio	30					
			Gastro	30					
			Hemato	30					
			Musc/skel	30					
			Hepatic	0.3	3	(dose-related hepatocyte enlargement and single-cell necrosis)			
			Renal	30					
			Endocr	30					
			Derm	30					
			Ocular	30					
			Bd Wt	30					

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
9	Rat (Sherman)	once 2-14 mo observation (GO)	Resp	1000			Kimbrough et al. 1978 (FF-1)
			Cardio	1000			
			Gastro	1000			
			Hepatic			1000 (vacuolation, necrosis, and fibrosis, porphyria, multinucleation)	
			Renal	1000			
			Endocr	1000			
			Bd Wt	1000			
10	Rat (Sherman)	once 15wk observation (GO)	Hepatic		500 M (vacuolation of hepatocytes)		Kimbrough et al. 1980 (FF-1)
			Bd Wt	500 M			
11	Rat (Sherman)	once 18-22mo observation (GO)	Resp	200 F			Kimbrough et al. 1981 (FF-1)
			Cardio	200 F			
			Hepatic		200 F (porphyrin accumulation)		
			Renal	200 F			
			Endocr	200 F			
			Derm	200 F			
			Bd Wt	200 F			

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
12	Rat (Sherman)	once 23mo observation (GO)	Resp	1000 F			Kimbrough et al. 1981 (FF-1)
			Cardio	1000 F			
			Gastro	1000 F			
			Musc/skel	1000 F			
			Hepatic		1000 F (hepatomegaly, hepatocyte enlargement and vacuolation, porphyrin accumulation)		
			Renal	1000 F			
			Endocr	1000 F			
			Ocular	1000 F			
			Bd Wt		1000 F (12% decreased body weight gain)		
13	Rat (Sprague-Dawley)	once 28d observation (GO)	Hepatic		1000 M (fatty changes in centrilobular hepatocytes)		Lee et al. 1975a (OBB)
			Bd Wt	1000 M			
14	Rat (Sprague-Dawley)	2 d 1x/d (GO)	Hepatic		3000 M (fatty changes in centrilobular hepatocytes)		Lee et al. 1975a (OBB)
			Bd Wt	1000 M			
15	Rat (Sprague-Dawley, Spartan)	once (GO)	Bd Wt	2000 F			Norris et al. 1975 (OBB)
16	Rat (Fischer 344)	10 d ad lib (F)	Hepatic		5 M (hepatomegaly and fatty changes in weanlings)		Raber and Carter 1986 (BP-6)
			Bd Wt	5 M			

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
17	Rat (Sprague-Dawley)	2 wk ad lib (F)	Hepatic	0.66 M	6.53 M (hyperplasia and fatty changes)		Waritz et al. 1977; Lee et al. 1975b (OBB)
			Renal	71 M			
			Endocr	71 M			
			Bd Wt	71 M			
18	Mouse (Swiss-Webster)	2 wk ad lib (F)	Hepatic	36 F			Cagen et al. 1977 (BP-6)
19	Mouse (Swiss/ IRC)	11 d ad lib (F)	Hepatic		130 F (focal areas of coagulative necrosis)		Corbett et al. 1975 (BP-6)
20	Mouse (Swiss/ IRC)	4-14 d ad lib (F)	Bd Wt			130 M (30% decreased body weight)	Corbett et al. 1978 (BP-6)
21	Mouse (Balb/c)	14 d ad lib (F)	Bd Wt			130 F (23% weight loss)	Fraker 1980 Fraker and Aust 1978 (BP-6)

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
22	Mouse (B6C3F1)	2 wk 5 d/wk 1x/d	Resp	30			Gupta et al. 1981
		(GO)	Cardio	30			(FF-1)
			Gastro	30			
			Hemato	30			
			Musc/skel	30			
			Hepatic	0.3	3 (dose-related increase in incidence of hepatocyte enlargement and single-cell necrosis)		
			Renal	30			
			Endocr	30			
			Ocular	30			
			Bd Wt	30			
			Other	30			
	Reproductive						
23	Rat (Sherman)	once 23mo observation			1000 F (9% increased incidence of uterine polyps)		Kimbrough et al. 1981
		(GO)					(FF-1)
24	Mouse (C57BL)	9 d Gd 6-15 1x/d		21 F		63 F (29% reduction in success of pregnancy)	Welsch and Morgan 1985
		(F)					(HBB)
	Developmental						
25	Rat (Wistar)	once Gd 6-14		40		200 (9.1-31.4% resorptions)	Beaudoin 1977
		(GO)					(BP-6)

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
26	Rat (Sprague-Dawley)	14 d Gd 7-20 (F)		5	50 (12% decrease in fetal body weight)		Corbett et al. 1975 (BP-6)
27	Rat (Sherman)	Gd 7-14 1x/d (GO)				200 (increased mortality and liver neoplasms in offspring)	Groce and Kimbrough 1984 (FF-1)
28	Rat (NS)	9 d Gd 7-15 1x/d (GO)			42.9 (12-20% decreased mean body weight in treated pups at post-parturition day 60)		Harris et al. 1978 (BP-6)
29	Rat (Sprague-Dawley, Iffa credo)	10 d Gd 6-15 1x/d (GO)		1000			Millischer et al. 1980 (DBB)
30	Rat (ChR-CD)	10 d Gd 6-15 ad lib (F)		9.1	86 (increased incidence of extra ribs)		Waritz et al. 1977 (OBB)
31	Mouse (Swiss/ IRC)	12 d Gd 7-18 (F)		5		50 (cleft palate)	Corbett et al. 1975 (BP-6)
	Cancer						
32	Rat (Sherman)	Gd 7-14 1x/d (GO)				200 (CEL: hepatocellular carcinoma in offspring)	Groce and Kimbrough 1984 (FF-1)

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
33	Rat (Sherman)	once (GO)				1000 F (CEL: hepatocellular carcinoma)	Kimbrough et al. 1981 (FF-1)
	Immuno/Lymphor						
34	Rat (Fisher 344/N)	10 d 5 d/wk 1x/d (GO)				1000 (atrophy of thymus; necrosis of splenic lymphoblasts)	Gupta and Moore 1979 (FF-1)
35	Mouse (Balb/c)	14 d ad lib (F)				130 F (suppressed antibody-mediated response to SRBC, thymic atrophy)	Fraker 1980 Fraker and Aust 1978 (BP-6)

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	INTERMEDIATE EXPOSURE						
	Death						
36	Rat (Fischer 344/N)	4.5 wk 5 d/wk 22 d (GO)				65[c] F (90-day LD50)	Gupta and Moore 1979 (FF-1)
						149 M (90-day LD50)	
37	Rat (Fischer 344/N)	25 wk 5 d/wk 1x/d (GO)				0.3 M (decreased mean survival time)	NTP 1983 (FF-1)
38	Mouse (B6C3F1)	25 wk 5 d/wk 1x/d (GO)				10 F (decreased mean survival time)	NTP 1983 (FF-1)
39	Gn pig (NS)	30 d ad lib (F)				4 M (4/6 died)	Sleight and Sanger 1976 (NS)
40	Gn pig (NS)	45 d ad lib (F)				2.0 F (7/8 died)	Vos and van Genderen 1973, 1974 (BP-6)
41	Mink (NS)	313 d ad lib (F)				0.47[c] M (LD50)	Aulerich and Ringer 1979 (FF-1)
						0.61 F (LD50)	

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	Systemic						
42	Monkey (Rhesus)	137 d ad lib (F)	Gastro		18 F (hyperplastic gastroenteritis)		Allen et al. 1978 (FF-1)
			Hemato		18 F (decreased RBC, PCV, and WBC)		
			Hepatic		18 F (enlarged hepatocytes, hyperplasia of bile duct epithelium, increased SGPT, decreased serum cholesterol)		
			Renal		18 F (hyperplasia of bladder epithelium)		
			Endocr			18 F (adrenal hemorrhage)	
			Derm		18 F (edema, atrophy, and squamous metaplasia of sebaceous glands)		
			Bd Wt			18 F (27% body weight loss)	

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
43	Monkey (Rhesus)	25-50 wk ad lib (F)	Cardio		0.73 M (enlarged heart at necropsy)		Allen et al. 1978; Lambrecht et al. 1978 (FF-1)
			Gastro			0.73 M (proliferation of mucosal cells, chronic inflammatory cells, severe ulcerative colitis)	
			Hemato		0.73 M (decreased PCV and total serum protein)		
			Hepatic		0.73 (enlarged hepatocytes with increased lipid droplets, increased SGPT, decreased serum cholesterol, hyperplasia of bile duct epithelium)		
			Derm			0.73 (edema and alopecia, keratinization of hair follicles and sebaceous glands)	
			Bd Wt			0.73 (34% weight loss in adult male, 0% weight gain in juvenile)	

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL: Less serious (mg/kg/day)	LOAEL: Serious (mg/kg/day)	Reference Chemical Form
44	Rat (Sprague-Dawley)	30 d ad lib (F)	Resp	10 M			Akoso et al. 1982a (BP-6)
			Cardio	10 M			
			Gastro	10 M			
			Hemato	10 M			
			Musc/skel	10 M			
			Hepatic		0.1 M (hepatocyte swelling, vacuolation)		
			Renal	10 M			
			Endocr	10 M			
			Derm	10 M			
			Ocular	10 M			
			Bd Wt	10 M			
45	Rat (Sprague-Dawley)	30 d ad lib (F)	Endocr		0.05 M (altered thyroid follicular ultrastructure)		Akoso et al. 1982b (BP-6)
46	Rat (Sprague-Dawley)	20 d 1x/d (GO)	Endocr		1 M (decreased serum thyroid hormone T4)		Allen-Rowland s et al. 1981 (NS)
47	Rat (Sprague-Dawley)	7 mo ad lib (F)	Endocr		0.45 F (decreased thyroid serum T3 and T4 hormones)		Byrne et al. 1987 (BP-6)
48	Rat (Sprague-Dawley)	5-7 mo ad lib (F)	Endocr	0.05 F	0.25 F (decreased adrenal serum corticosterone B, DHEA and DHS hormones)		Byrne et al. 1988 (BP-6)

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
49	Rat (Sprague-Dawley)	20 d 1x/d (GO)	Endocr	6 M			Castracane et al. 1982 (NS)
50	Rat (Sprague-Dawley)	28 d ad lib (F)	Resp	2 M			Chu et al. 1980 (BP-6)
			Cardio	2 M			
			Gastro	2 M			
			Hemato	2 M			
			Hepatic		2 M (increased liver weight, increased liver microsomal enzymes, fatty degeneration of liver)		
			Renal	2 M			
			Endocr		2 M (reduction of follicular size and colloid density and exfoliation of epithelium in thyroid)		
			Derm	2 M			
			Bd Wt	2 M			
51	Rat (Sprague-Dawley)	82 d ad lib (F)	Hepatic		0.5 M (bile duct hyperplasia)		Darjono et al. 1983 (BP-6)
			Ocular		5 M (xerophthalmia)		
			Bd Wt	5 M			

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
52	Rat (Fischer 344/N)	4.5 wk 5 d/wk 1x/d	Cardio	1000					Gupta and Moore 1979
		(GO)	Gastro	1000					(FF-1)
			Hemato		30	(decreased hemoglobin, PCV, and platelet count)			
			Hepatic		30	(hepatocyte enlargement, fatty infiltration and multinucleation, porphyrin accumulation)			
			Renal		30	(dilation of Bowman's capsule with serous fluid)			
			Endocr		30	(unspecified altered thyroid histology)			
			Bd Wt		30	(19% decreased body weight gain)	100	(emaciation)	
53	Rat (Fischer 344/N)	30 d 5 d/wk 1x/d	Resp	30					Gupta et al. 1981
		(GO)	Cardio	30					(FF-1)
			Gastro	30					
			Hemato	30					
			Musc/skel	30					
			Hepatic	0.3	3.0	(increased liver weight, hepatocyte swelling, and necrosis)			
			Renal	30					
			Bd Wt	3	30	(significant decrease in body weight)			

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
54	Rat (Holtz-man)	5 wk ad lib (F)	Hepatic	0.25 M	2.5 M (hepatocyte hypertrophy and degeneration)		Kasza et al. 1978 (BP-6)
55	Rat (Holtz-man)	5 wk ad lib (F)	Endocr		0.25 M (colloid droplets, abnormal microvilli and other changes in thyroid follicle ultrastructure)		Kasza et al. 1978 (BP-6)
56	Rat (Fischer 344)	6 mo 5 d/wk (GO)	Hemato		10 F (increased white blood cell count)		Luster et al. 1980 (FF-1)
			Bd Wt	1.0 F	3.0 F (15% decreased weight gain)		
57	Rat (Sprague-Dawley)	3 mo ad lib (F)	Hepatic		5 F (enlarged and vacuolated hepatocytes, focal necrosis)		McCormack et al. 1978 (BP-6)
			Renal		5 F (degenerative changes in glomeruli)		
			Bd Wt		5 F (10% decreased body weight gain)		
58	Rat (Sprague-Dawley CFY)	13 wk (F)	Gastro	100			Millischer et al. 1980 (DBB)
			Hemato	100			
			Hepatic	25	100 (11% increased liver weight, hepatocyte hypertrophy and vacuolization, slightly increased liver lipids)		
			Renal	100			
			Bd Wt	100			

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
59	Rat (Sprague-Dawley)	30 d ad lib (F)	Cardio	800 M			Norris et al. 1975 (OBB)
			Hemato	80 M	800 M (decreased PCV and RBC counts)		
			Hepatic		8 M (enlargement and vacuolation)		
			Renal		8 M (hyaline degenerative changes)		
			Endocr		8 M (thyroid hyperplasia)		
			Bd Wt	800 M			
60	Rat (Sprague-Dawley)	8 mo ad lib (F)	Cardio	1			Norris et al. 1975 (OBB)
			Hemato	1			
			Hepatic	1			
			Renal	1			
			Endocr	1			
			Bd Wt	1			

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
61	Rat (Fischer 344/N)	25 wk 5 d/wk 1x/d (GO)	Resp	10			NTP 1983 (FF-1)
			Cardio	10			
			Gastro	0.3	1 (gastric ulcers)		
			Hemato	0.1	0.3 (decreased hemoglobin, MCH, PCV, and MCV)		
			Musc/skel	10			
			Hepatic	0.1	0.3 (lipid accumulation; increased atypical foci; porphyrin accumulation)		
			Renal	0.3		1 (chronic progressive nephropathy)	
			Endocr	0.1	0.3 (decreased serum thyroid T4 hormone)		
			Ocular	10			
			Bd Wt	10			
62	Rat (Sprague-Dawley)	7 mo ad lib (F)	Endocr	2.5 F			Sepkovic and Byrne 1984 (HBB)
63	Rat (Sprague-Dawley)	7 mo ad lib (F)	Hepatic	2.5 F			Sepkovic and Byrne 1984 (OBB)
			Endocr	2.5 F			

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
64	Rat (Sprague-Dawley)	30 d ad lib (F)	Resp	50 M			Sleight and Sanger 1976 (NS)
			Cardio	50 M			
			Gastro	50 M			
			Hemato	50 M			
			Musc/skel	50 M			
			Hepatic		1 M (hepatocyte vacuolation)		
			Renal	10 M	50 M (unquantified, but significantly increased BUN)		
			Endocr	50 M			
			Bd Wt	10 M	50 M (16% decreased weight gain)		
65	Rat (Sprague-Dawley)	30 d ad lib (F)	Resp	5 M			Sleight et al. 1978 (BP-6)
			Cardio	5 M			
			Gastro	5 M			
			Hemato	5 M			
			Hepatic	0.5 M	5 M (hepatocyte swelling and vacuolation)		
			Renal	5 M			
			Endocr	0.5 M	5 M (hyperplasia of thyroid follicular epithelium)		
			Bd Wt	0.5 M	5 M (27-36% reduced body weight gain)		

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
66	Rat (Sprague-Dawley)	4 wk ad lib (F)	Hepatic	0.66 M	6.53 M (hyperplasia and progressive lipid changes)		Waritz et al. 1977; Lee et al. 1975b (OBB)
			Renal	71 M			
			Endocr	71 M			
			Bd Wt	71 M			
67	Mouse (Balb/c)	30 d ad lib (F)	Bd Wt	13 F			Fraker 1980 Fraker and Aust 1978 (BP-6)
68	Mouse (B6C3F1)	30 d 5 d/wk 1x/d (GO)	Resp	30			Gupta et al. 1981 (FF-1)
			Cardio	30			
			Gastro	30			
			Hemato		30 F (decreased PCV)		
			Musc/skel	30			
			Hepatic	0.3	3 (hepatocyte enlargement and necrosis)		
			Renal	30			
			Ocular	30			
			Bd Wt	3	30 M (significant decrease in weight gain)		
			Other	30			

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
69	Mouse (Balb/ cBYJ)	6 wk ad lib (F)	Resp	21.7 M			Loose et al. 1981 (FF-1)
			Cardio	21.7 M			
			Hepatic	0.65 M		21.7 M (hepatocellular necrosis and vacuolation)	
			Renal	21.7 M			
			Endocr	0.65 M			
			Bd Wt	0.65 M		21.7 M (33% reduction in body weight)	
70	Mouse (B6C3F1)	6 mo 5 d/wk (GO)	Hemato	10			Luster et al. 1980 (FF-1)
			Endocr		10 (increased adrenal weight gain)		
			Bd Wt	10			

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL	LOAEL				Reference Chemical Form
					Less serious		Serious		
71	Mouse (B6C3F1)	25 wk 5 d/wk 1x/d (GO)	Resp	10					NTP 1983 (FF-1)
			Cardio	10					
			Gastro	10					
			Hemato	0.1	0.3	(decreased erythrocyte count and MCV)			
			Musc/skel	10					
			Hepatic	0.1	0.3	(increased liver weight, SGOT and porphyrin accumulation)			
			Renal	10					
			Endocr	10					
			Ocular	10					
			Bd Wt	3 M			10 M	(25% decreased body weight gain)	
72	Gn pig (NS)	30 d ad lib (F)	Resp	20 M					Sleight and Sanger 1976 (NS)
			Hepatic		0.04 M	(vacuolation and fatty changes)			
			Bd Wt	0.4 M			4 M	(severe weight loss prior to death)	

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
73	Pig (NS)	16 wk ad lib (F)	Cardio	8			Ku et al. 1978 (NS)
			Gastro	1	8 (gross hyperplasia glandular stomach)		
			Hemato	8			
			Hepatic		1 (LDH increased)		
			Renal	8			
			Endocr		8 (increased adrenal weight)		
			Derm	1	8 (dermatosis)		
			Bd Wt		1 (12.9% reduced weight gain and food intake)		
74	Pig (NS)	12 wk Gwk 8-ppwk 4 ad lib (F)	Hepatic	0.125 F	1.25 F (fatty changes and necrosis)		Werner and Sleight 1981 (BP-6)
			Endocr	1.25 F	2.5 F (significant decrease in thyroid serum T3 and T4 hormones)		
75	Mink (NS)	313 d ad lib (F)	Cardio	2.4			Aulerich and Ringer 1979 Ringer and Aulerich 1981 (FF-1)
			Hepatic		0.24 F (48% increased relative liver weight, fatty infiltration)		
			Renal	2.4			
			Bd Wt		0.39 F (14% decreased prebreeding body weight gain)	1.86 F (up to 19% mean body weight loss prior to death)	

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	Neurological						
76	Rat (NS)	6 mo 5 d/wk 1x/d (GO)			10 (decreased limb strength)		Cabe and Tilson 1978 (FF-1)
77	Rat (Sprague-Dawley Holtz-man)	4 wk 5 d/wk 1x/d (G)		3 M		6 M (decreased motor activity)	Geller et al. 1979 (FF-1)
78	Rat (Sprague-Dawley)	40d Gd 6-Ppd 24 (F)		0.2	2 (delayed acquisition of locomotion and reduced open field activity in offspring)		Henck et al. 1994 (BP-6)
79	Rat (Fischer 344/N)	6 mo 5 d/wk (GO)				3 (decreased motor activity, grip strength, and startle responsiveness)	Tilson and Cabe 1979 (FF-1)
80	Rat (Fischer 344/N)	4 wk 5 d/wk 1x/d (GO)				30 (decreased open field motor activity and grip strength)	Tilson and Cabe 1979 (FF-1)
	Reproductive						
81	Monkey (Rhesus)	25-50 wk ad lib (F)			0.73 M (hypoactive seminiferous tubules)		Allen et al. 1978; Lambrecht et al. 1978 (FF-1)

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
82	Monkey (Rhesus)	6 mo ad lib (F)				0.012 F (increased menstrual cycle duration in 4/7; implantation bleeding in 2/7)	Lambrecht et al. 1978; Allen et al. 1978; 1979 (FF-1)
83	Rat (Wistar)	15 d Gd 0-14 8x (GO)		14.3 F		28.6 F (no implantations in 2/5 rats)	Beaudoin 1979 (BP-6)
84	Rat (Fischer 344/N)	4-5 wk 5 d/wk 22 d (GO)		30 M 1000 F	100 M (squamous metaplasia, hyperplasia, and necrosis in epithelium of ductus deferens)		Gupta and Moore 1979 (FF-1)
85	Rat (Sprague-Dawley)	42 d Gd 8-ppd 28 ad lib (F)		5 F			McCormack et al. 1981 (BP-6)
86	Mouse (B6C3F1)	4-5 wk 5 d/wk 1x/d (GO)		30			Gupta et al. 1981 (FF-1)
87	Mink (NS)	313 d ad lib (F)		0.24	0.39 F (10% reduction in body weight)		Aulerich and Ringer 1979 (FF-1)

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	Developmental						
88	Rat (Wistar)	15 d Gd 0-14 8x (GO)		2.9		14.3 (increased resorptions)	Beaudoin (BP-6)
89	Rat (Holtz-man Sprague-Dawley)	4 wk 5 d/wk (G)		5			Geller et al. 1985 (FF-1)
90	Rat (Sprague-Dawley)	40 d Gd 6- ppd24 ad lib (F)				0.2 (deficits in learning behavior in offspring, 6 months after prenatal and lactational exposure)	Henck and Rech 1986 (BP-6)
91	Rat (Sprague-Dawley)	40d Gd 6-Ppd 24 (F)			0.2 M (reduced crown-rump length)		Henck et al. 1994 (BP-6)
92	Rat (Sprague-Dawley)	42 d Gd 8-ppd 28 ad lib (F)			0.5 (increased liver weight, hepatocyte vacuolation, decreased hepatic vitamin A content in F1 but not F2)	5 (decreased pup survival during lactation in F1)	McCormack et al. 1981 (BP-6)
93	Rat (Sprague-Dawley)	42-126 d Gd 8-ppd 28-112 ad lib (F)			5 (20% decrease in pup body weight gain, 50% decreased hepatic vitamin A, 256-285% decreased urinary uro- and coproporphyrins in pups)		McCormack et al. 1982a (BP-6)

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
94	Rat (Sprague-Dawley)	37 d Gd 0-ppd 15 ad lib (F)			2.5	(decreased body weight, increased relative liver weight, and decreased serum T4 in offspring)			Meserve et al. 1992 (BP-6)
95	Rat (Fischer 344/N)	77 d Gd 0-ppd 56 ad lib (F)			0.5	(hepatic vacuolization and altered foci in pups)			NTP 1992 Chhabra et al. 1993 (FF-1)
96	Mouse (B6C3F1)	42 d Gd 0- weaning 1x/2d (GO)		2	3	(decreased hematocrit in offspring)	10	(early postnatal death; no details provided)	Luster et al. 1980 (FF-1)
97	Mouse (B6C3F1)	77 d Gd 0- ppd 56 ad lib (F)			1.5	(hepatic cytomegaly and altered foci in pups)			NTP 1992 Chhabra et al. 1993 (FF-1)
98	Mouse (C57B1/6)	Gd 0-ppd 21 1x/2d (GO)			3	(performance deficits in offspring in a learned task)			Tilson 1992 (FF-1)
99	Pig (NS)	12 wk Gwk 8- ppwk 4 ad lib (F)		0.125			1.25	(increased relative liver weight, decreased serum thyroid hormone levels, and slight thyroid hyperplasia in offspring)	Werner and Sleight 1981 (BP-6)
100	Mink (NS)	313 d (F)			0.155	(decreased birth and 4-week weights in kits)			Aulerich and Ringer 1979 Ringer and Aulerich 1981 (FF-1)

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	Cancer						
101	Rat (Sherman)	4 mo 12 x (GO)				100 (CEL: hepatocellular carcinoma)	Kimbrough et al. 1981 (FF-1)
102	Rat (Fischer 344/N)	25 wk 5 d/wk 1x/d (GO)				3 (CEL: hepatocellular carcinoma)	NTP 1983 (FF-1)
103	Mouse (B6C3F1)	25 wk 5 d/wk 1x/d (GO)				10 (CEL: hepatocellular carcinomas)	NTP 1983 (FF-1)
104	Mouse (B6C3F1)	77 d Gd 0-ppd 56 ad lib (F)				1.5 (CEL: hepatocullular adenoma and carcinoma in offspring)	NTP 1992 Chhabra et al. 1993 (FF-1)
	Immuno/Lymphor						
105	Rat (Fischer 344)	5 wk 5 d/wk (GO)		0.03 M	3.0 M (decreased lymphocytic response to mitogen stimulation; decrease in absolute and relative thymus weight)		Luster et al. 1978 (FF-1)
106	Rat (Fischer 344)	6 mo 5 d/wk 1x/d (GO)		1 F	3 F (decreased lymphoproliferative responses and decreased delayed hypersensitivity responses)		Luster et al. 1980 (FF-1)

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
107	Mouse (Balb/c)	30 d ad lib (F)		0.13 F	1.3 F (reduced antibody mediated response to SRBC and 21% reduction in thymus weight)		Fraker 1980 Fraker and Aust 1978 (BP-6)
108	Mouse (Balb/ cBYJ)	6 wk ad lib (F)		0.65 M		21.7 M (increased lethality due to endotoxin challenge)	Loose et al. 1981 (FF-1)
109	Mouse (B6C3F1)	6 mo 5 d/wk 1x/d (GO)		3.0		10 (increased lethality due to infection with L monocytogenes; decreased response to mitogen stimulation)	Luster et al. 1980 (FF-1)
110	Gn pig (NS)	45 d ad lib (F)			0.4 F (reduced antitoxin titers following toxoid challenge)	4.0 F (thymic atrophy and follicular depletion in spleen)	Vos and van Genderen 1973, 1974 (BP-6)
111	Pig (NS)	12 wk Gwk 8- ppwk 4 ad lib (F)		1.25 F	2.5 F (reduced lymphocyte response to mitogen stimulation)		Howard et al. 1980 (BP-6)

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	CHRONIC EXPOSURE						
	Death						
112	Rat (Fischer 344/N)	115 wks Gd 0-ppd 56 (weaning) 104 wks post-weaning ad lib (F)				0.5 M (18% decreased survival)	NTP 1992 Chhabra et al. 1993 (FF-1)
113	Mouse (B6C3F1)	116 wks Gd 0-ppd 56 (weaning) 105wks post-weaning ad lib (F)				1.3 F (44% decreased survival)	NTP 1992 Chhabra et al. 1993 (FF-1)
	Systemic						
114	Monkey (Rhesus)	66 wk ad lib (F)	Hemato	0.012			Lambrecht et al. 1978 (FF-1)
			Bd Wt		0.012 (7.4% weight loss)		

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
115	Rat (Fischer 344/N)	115 wks Gd 0-ppd 56; 104 wks post-weaning (F)	Resp	1.5			NTP 1992 Chhabra et al. 1993 (FF-1)
			Cardio	1.5			
			Gastro	0.5	1.5 M (forestomach hyperplasia, inflammation, ulceration)		
			Hemato	0.5	1.5 F (mild anemia)		
			Musc/skel	1.5			
			Hepatic		0.5 (hypertrophy, vacuolation, altered foci, increased serum cholesterol, decreased serum triglycerides)		
			Renal	1.5			
			Endocr	1.5			
			Derm	1.5			
			Bd Wt		0.5 (11-18% decreased final body weight)		

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
116	Mouse (B6C3F1)	116 wks Gd 0-ppd 56 (weaning) 105 wks post-weaning ad lib (F)	Resp	3.9			NTP 1992 Chhabra et al. 1993 (FF-1)
			Cardio	3.9			
			Gastro	3.9			
			Musc/skel	3.9			
			Hepatic		1.3 (hypertrophy, vacuolization, single-cell necrosis, altered foci, bile duct hyperplasia)		
			Renal	1.3		3.9 (increased chronic nephropathy)	
			Endocr		1.3 (thyroid follicular cell hyperplasia)		
			Derm	3.9			
			Bd Wt	3.9			
Reproductive							
117	Rat (Fischer 344/N)	115 wks Gd 0- ppd-56 (weaning) 104 wks post-weaning ad lib (F)		0.5 F 1.5 M	1.5 F (cystic endometrial hyperplasia)		NTP 1992 Chhabra et al. 1993 (FF-1)

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form
118	Mouse (B6C3F1)	116 wks Gd 0-ppd 56 (weaning) 105 wks post-weaning ad lib (F)		3.9				NTP 1992 Chhabra et al. 1993 (FF-1)
	Developmental							
119	Monkey (Rhesus)	359-469 d ad lib (F)				0.012	(1/7 fetuses were aborted, 1/7 fetuses stillborn, 12% decreased birth weight and 22% decreased postnatal weight gain in 4/7 survivors)	Lambrecht et al. 1978; Allen et al. 1978; Allen et al. 1979 (FF-1)
	Cancer							
120	Rat (Fischer 344/N)	115 wks Gd 0- ppd 56 104 wks post-weaning (F)				0.5	(CEL: hepatocellular adenoma and carcinoma)	NTP 1992 Chhabra et al. 1993 (FF-1)
						1.5	(CEL: leukemia)	
121	Mouse (B6C3F1)	116 wks Gd 0- ppd 56 105wks post-weaning (F)				1.3	(CEL: hepatocellular adenoma and carcinoma)	NTP 1992 Chhabra et al. 1993 (FF-1)
						3.9	(thyroid follicular cell adenoma)	

Table 3-1. Levels of Significant Exposure to PBBs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
Immuno/Lymphor							
122	Rat (Fischer 344/N)	115 wks Gd 0- ppd 56 (weaning) 104 wks post-weaning ad lib (F)		0.5 M	1.5 M (splenic fibrosis)		NTP 1992 Chhabra et al. 1993 (FF-1)
123	Mouse (B6C3F1)	116 wks Gd 0-ppd 56 (weaning) 105 wks post-weaning ad lib (F)		1.3	3.9 (increased splenic hematopoiesis)		NTP 1992 Chhabra et al. 1993 (FF-1)

[a]The number corresponds to entries in Figure 3-1.

[b]Used to derive an acute-duration oral minimal risk level (MRL) of 0.01 mg/kg/day. The MRL was derived by dividing the NOAEL by an uncertainty factor of 100 (10 for extrapolation from animals to humans, 10 for human variability).

[c] Differences in levels of helath effects and cancer effects between male and females are not indicated in Figure 3-1. Where such differences exist, only the levels of effect fot the most sensitive gender are presented.

ad lib - ad libitum; Bd Wt = body weight; BP-6 = FireMaster BP-6; BUN = blood urea nitrogen; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); DBB = decabromobiphenyl; DHEA = dehydroepiandrosterone; DHS = dehydroepiandrosterone sulfate; Endocr = endocrine; (F) = feed; F = Female; FF-1= FireMaster FF-1; G) = gavage; Gastro = gastrointestinal; gd = gestational day; Gn pig = guinea pig; (GO) = gavage in oil; HBB = hexabromobiphenyl; hemato = hematological; hr = hour(s); LDH = lactic dyhydrogenase; LOAEL = lowest-observed-adverse-effect level; M = male; MCH = mean corpuscular hemoglobin; MCV = mean corpuscular volume; min = minute(s); mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; OBB = Octabromobiphenyl; PCV = packed cell volume; pmd = pre-mating day; pnd = post-natal day; occup = occupational; RBC = red blood cell; Resp = respiratory; SGOT = serum glutamic oxaloacetic transaminase; SGPT = serum glutamic pyruvic transaminase ; SRBC = sheep red blood cells; x = time(s); (W) = drinking water; WBC = white blood cell; wk = week(s)

Figure 3-1. Levels of Significant Exposure to PBBs - Oral

Acute (≤14 days)

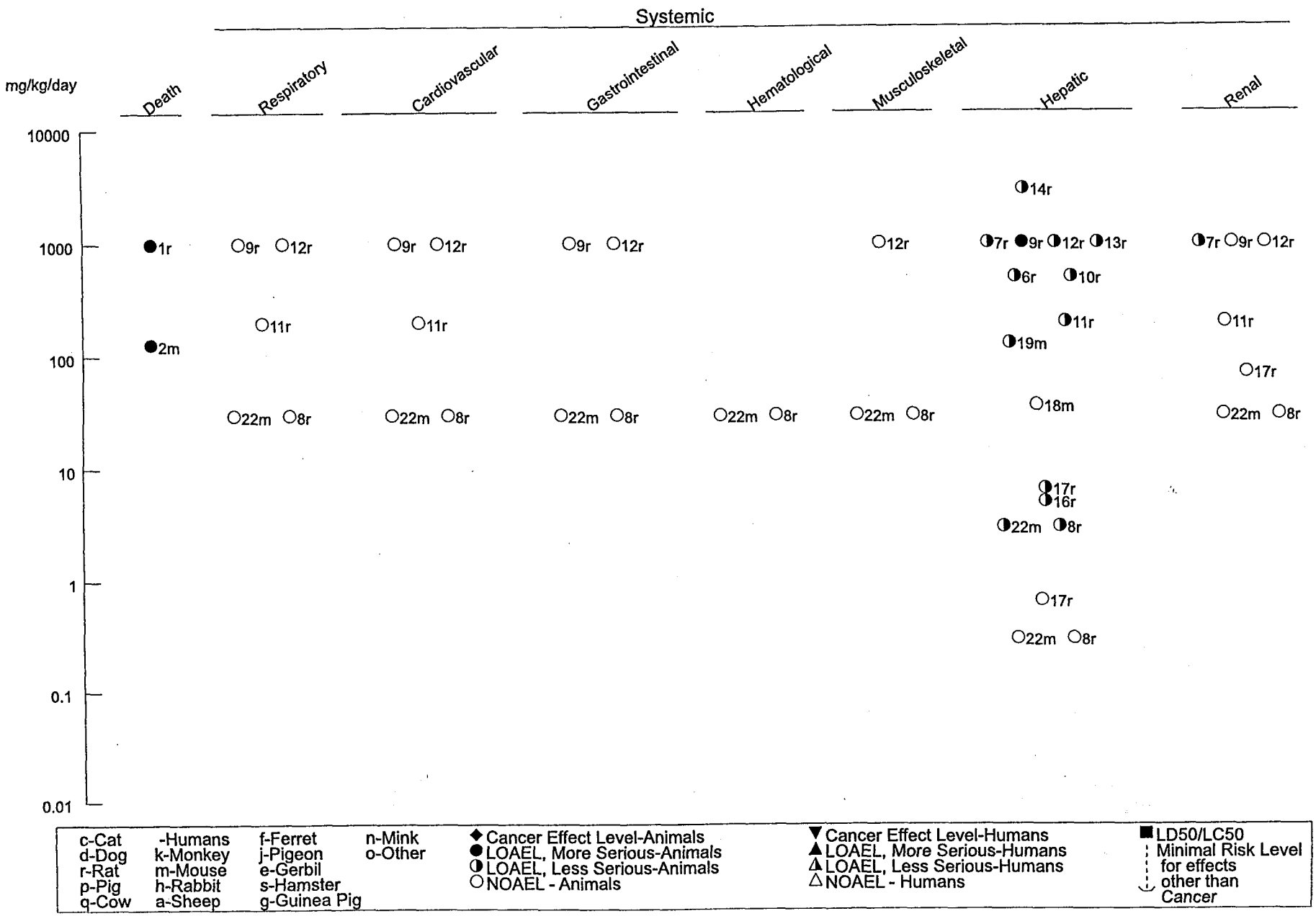


Figure 3-1. Levels of Significant Exposure to PBBs - Oral (*Continued*)

Acute (≤14 days)

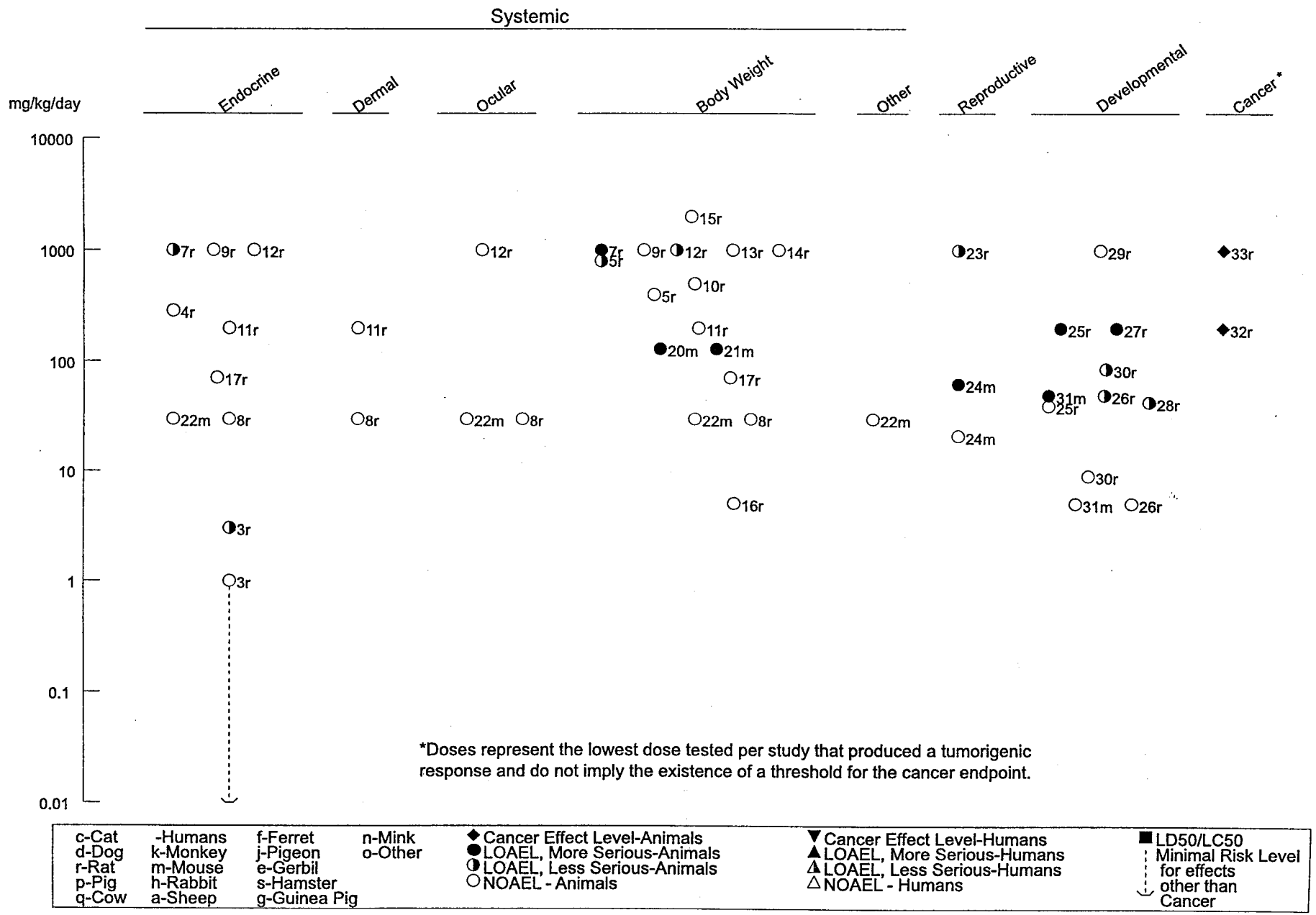


Figure 3-1. Levels of Significant Exposure to PBBs - Oral (*Continued*)

Intermediate (15-364 days)

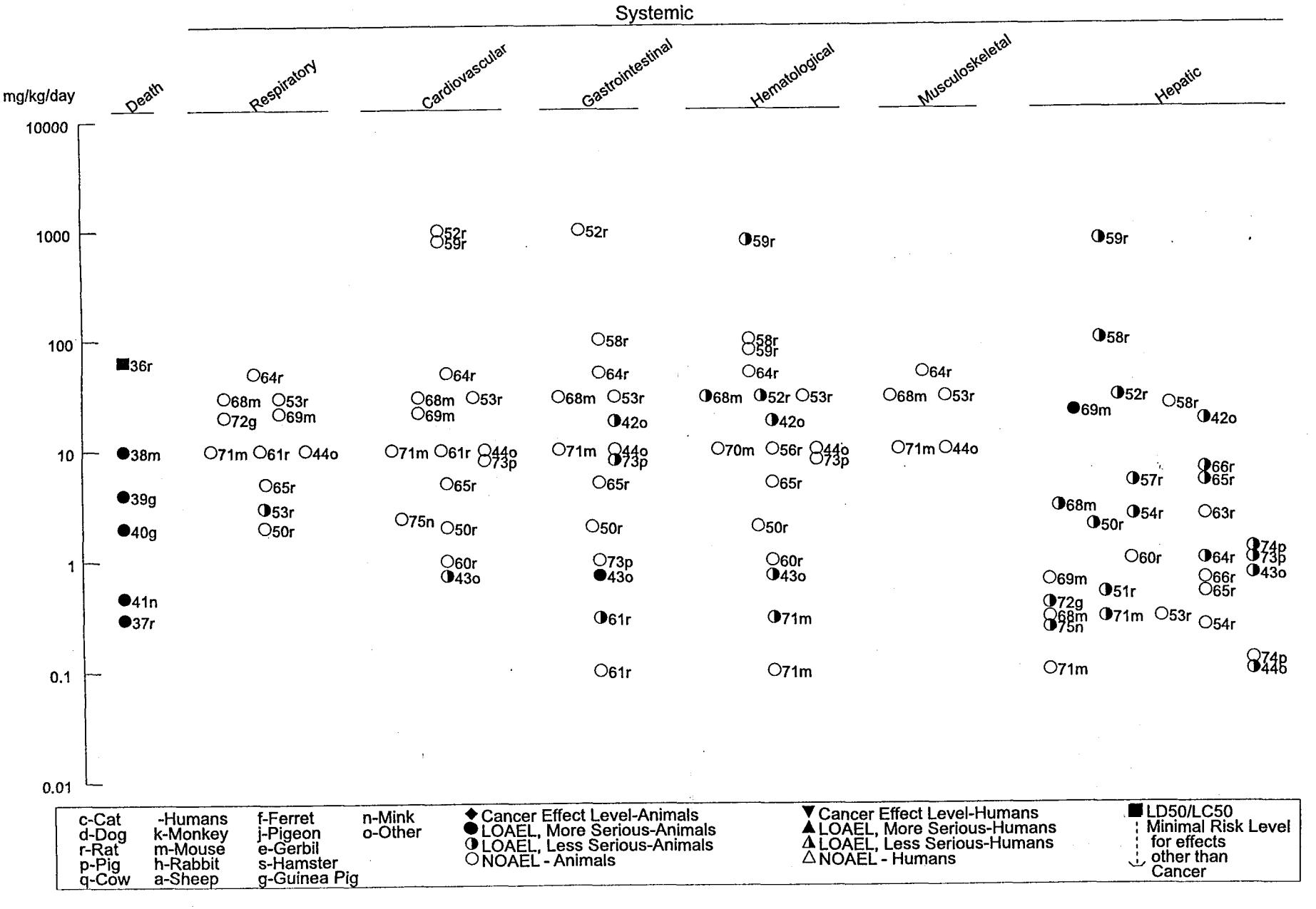


Figure 3-1. Levels of Significant Exposure to PBBs - Oral (*Continued*)

Intermediate (15-364 days)

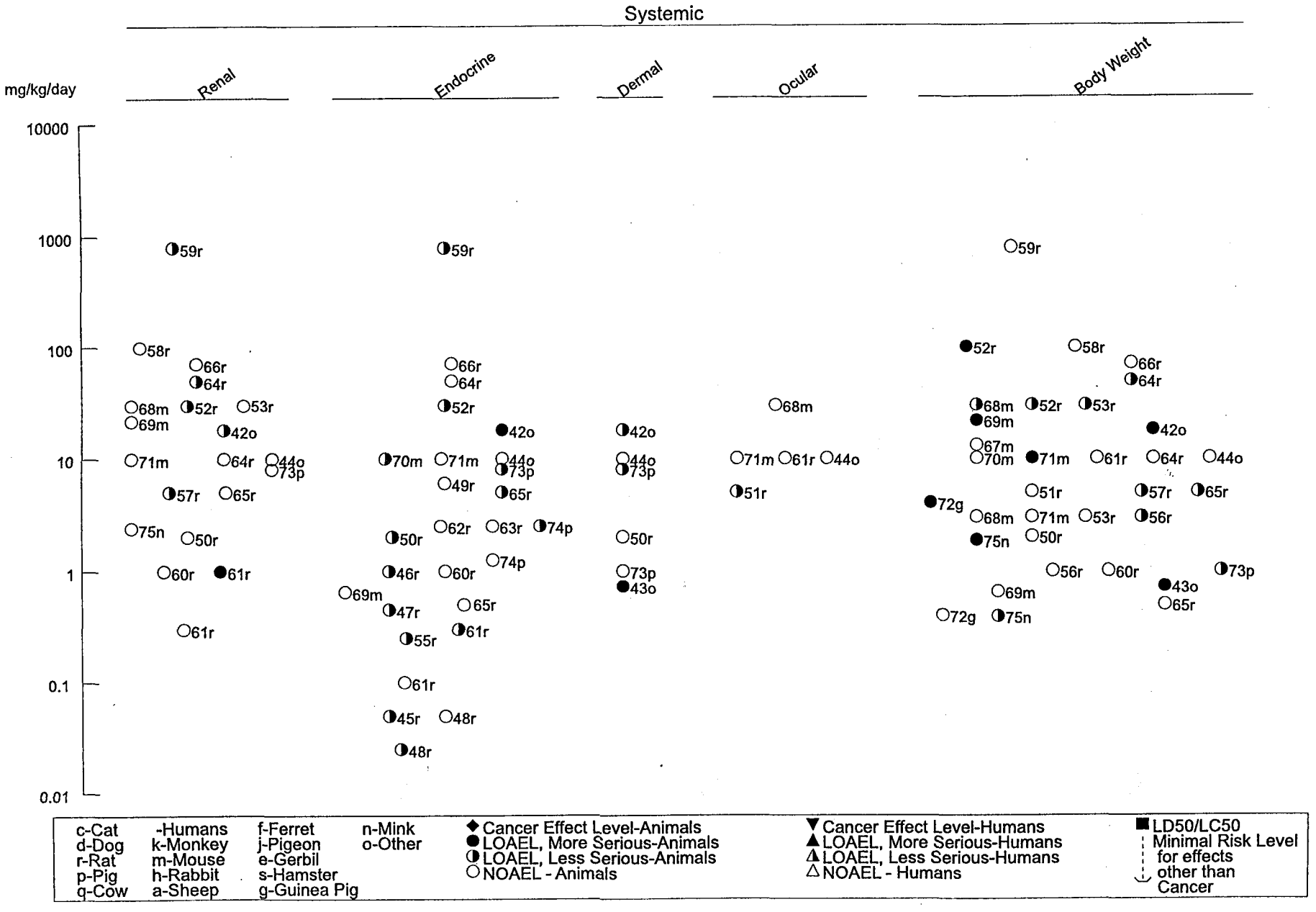


Figure 3-1. Levels of Significant Exposure to PBBs - Oral (*Continued*)

Intermediate (15-364 days)

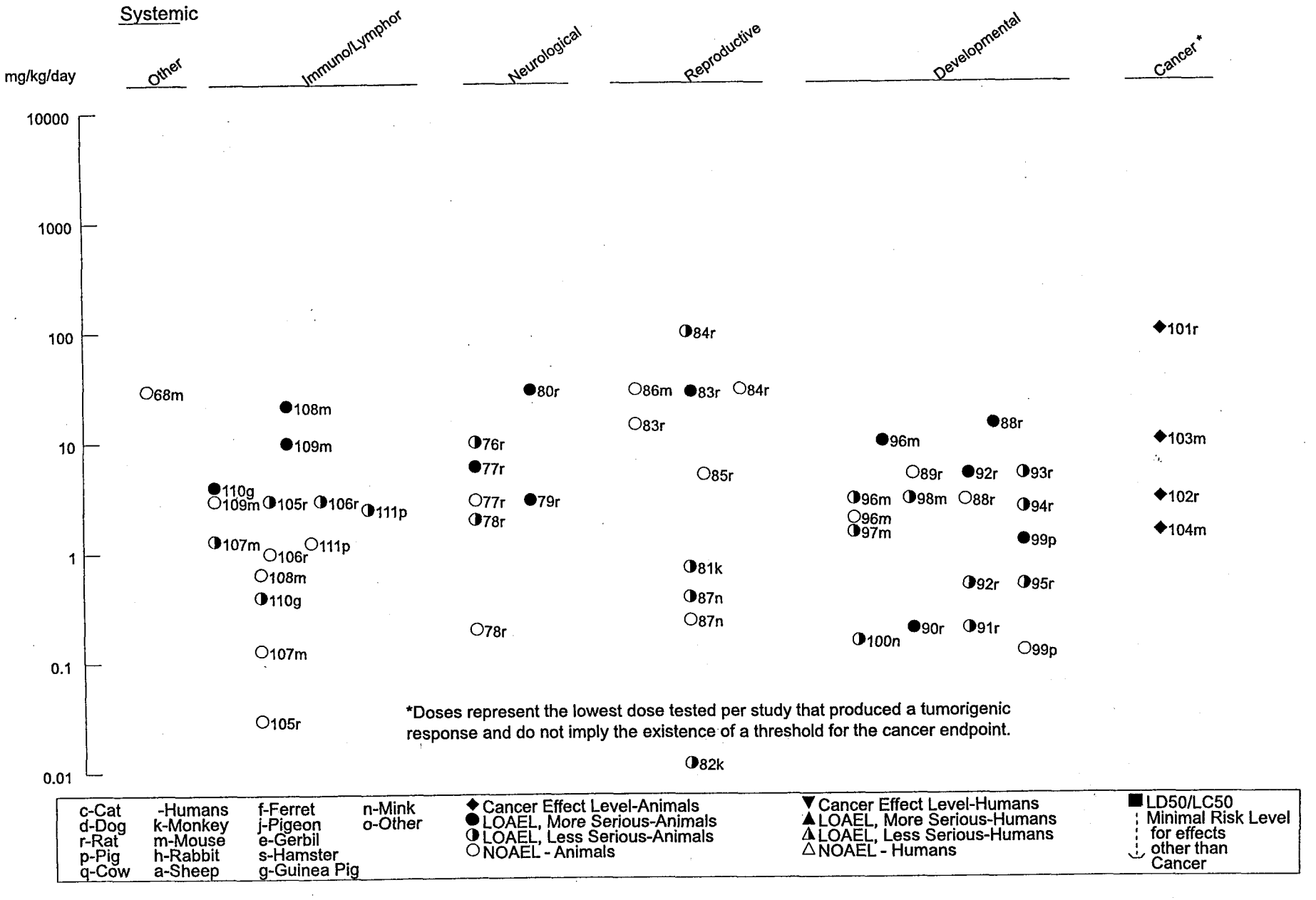
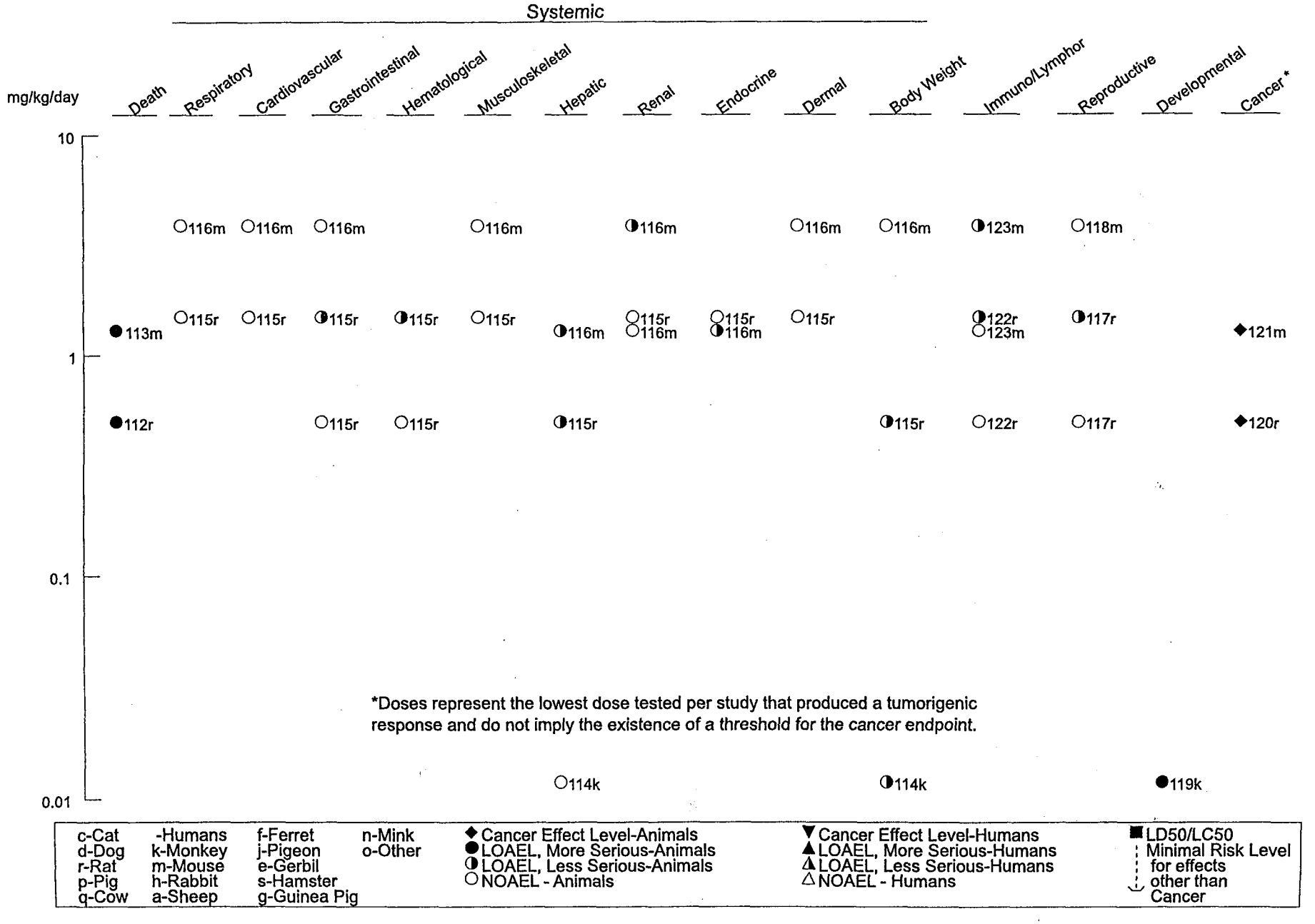


Figure 3-1. Levels of Significant Exposure to PBBs - Oral (*Continued*)

Chronic (≥365 days)



whereas doses #500 mg/kg caused no mortality (IRDC 1975b). No deaths occurred in rats exposed to pentaBDE in estimated dietary doses of #90 mg/kg/day for 28 days (IRDC 1976) or #100 mg/kg/day for 90 days (WIL Research Laboratories 1984).

The LD_{50} and LOAEL values for death in the acute-duration pentaBDE studies in rats are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

The systemic effects in humans and animals following oral exposure to PBBs and PBDEs are described below. The highest NOAEL and all LOAEL values from each reliable study for systemic end points in each species and duration category are recorded in Tables 3-1 and 3-2 and plotted in Figures 3-1 and 3-2.

Respiratory Effects.

Polybrominated Biphenyls. No studies were located regarding respiratory effects in humans after oral exposure to PBBs.

The preponderance of data does not indicate that PBBs are respiratory system toxicants in animals, even at doses sufficient to cause death. No exposure-related histological changes were observed in the lungs or trachea of rats that were administered FireMaster FF-1 in a single dose of 200 or 1,000 mg/kg and observed for 2–23 months posttreatment (Kimbrough et al. 1978b, 1981). Rats and mice exposed to #30 mg/kg/day FireMaster FF-1 for 2 weeks also showed no histological alterations in the lung, trachea, or nasal turbinates (Gupta et al. 1981). Information on acute-duration respiratory effects in other species was not located.

In intermediate- and chronic-duration studies with rats, histology of the lung, trachea, or nasal turbinate was not altered by FireMaster FF-1 or FireMaster BP-6 dosages of #30 mg/kg/day by gavage for 30 days (Gupta et al. 1981), #10 mg/kg/day in the diet for 30 days (Akoso et al. 1982a; Sleight et al. 1978), #10 mg/kg/day by gavage for 25 weeks (NTP 1983), or #1.5 mg/kg/day in the diet for up to 104 weeks (NTP 1992). Rat lung histology also was not affected by exposure to 50 mg/kg/day of an unspecified PBB mixture in the feed for 30 days (Sleight and Sanger 1976). In studies with mice, FireMaster FF-1 produced no histopathological changes in the lungs, trachea, or nasal turbinates following gavage

Table 3-2. Levels of Significant Exposure to PBDEs - Oral

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg)	LOAEL Less serious (mg/kg)	LOAEL Serious (mg/kg)		Reference Chemical Form
	ACUTE EXPOSURE							
	Death							
1	Rat (Wistar)	once (GO)				6200	(44-day LD50)	British Industrial Biological Research Association 1977 PentaBDE
2	Rat Spartan	once (GO)				5000	(4/5 died)	IRCD 1975b PentaBDE
3	Rat (Sprague-Dawley)	once (GO)				5000	(14-day LD50)	Pharmakon Research International Inc. 1984 PentaBDE
	Systemic							
4	Rat (Sprague-Dawley)	14 d 1x/d (GO)	Hepatic	95.9 M				Carlson 1980b DecaBDE
5	Rat Spartan	once (GO)	Bd Wt	5000				IRCD 1974 DecaBDE
6	Rat (Fischer- 344)	14d 1x/d (F)	Bd Wt	16,000				NTP 1986 DecaBDE
7	Rat (Long-Evans)	4 d 1x/d (GO)	Endocr	100				Zhou et al. 2001 DecaBDE

Table 3-2. Levels of Significant Exposure to PBDEs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL			Reference Chemical Form
					Less serious (mg/kg/day)		Serious (mg/kg/day)	
8	Mouse (B6C3F1)	14d 1x/d (F)	Bd Wt	19,000				NTP 1986 DecaBDE
9	Rat (Sprague-Dawley)	14 d 1x/d (GO)	Hepatic	76.6 M				Carlson, 1980b OctaBDE
10	Rat Spartan	once (GO)	Bd Wt	5000				IRCD 1975a OctaBDE
11	Rat (CD)	10 d Gd 6-15 (GO)	Bd Wt	25				Life Science Research Israel Ltd. (1987) OctaBDE
12	Rat (CD)	10 d Gd 6-15 (GO)	Bd Wt	25	50	(40% reduced maternal body weight gain)		WIL Research Laboratories 1986 OctaBDE
13	Rat (Long-Evans)	4 d 1x/d (GO)	Endocr	3	10	(reduced serum T4)		Zhou et al. 2001 OctaBDE
14	Rat (CD)	10 d Gd 6-15 (GO)	Bd Wt	10	100	(30% reduced maternal body weight gain)		Argus Research Laboratories 1985a PentaBDE
15	Rat (Sprague-Dawley)	14 d 1x/d (GO)	Hepatic	56.4 M				Carlson, 1980b PentaBDE

Table 3-2. Levels of Significant Exposure to PBDEs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
16	Rat (Sprague-Dawley)	14 d 1x/d (GO)	Endocr		18 (reduced serum T4)		Darnerud and Sinjari 1996 PentaBDE
17	Rat (Sprague-Dawley)	14 d 1x/d (GO)	Hepatic		18 (reduced liver vitamin A)		Hallgren et al. 2001 PentaBDE
			Endocr		18 (reduced serum T4)		
18	Rat Spartan	once (GO)	Bd Wt	500			IRCD 1975b PentaBDE
19	Rat (Long-Evans)	4 d 1x/d (GO)	Endocr	10	30 (reduced serum T4)		Zhou et al. 2001 PentaBDE
20	Mouse (C57BL/6N)	14 d 1x/d (GO)	Endocr		18 (reduced serum T4)		Darnerud and Sinjari 1996 PentaBDE
21	Mouse C57BL/6J	once (GO)	Hepatic	500			Fowles et al. 1994 PentaBDE
			Endocr	100	500 (reduced serum T4)		
22	Mouse (C57BL/6N)	14d 1x/d (GO)	Hepatic	72			Fowles et al. 1994 PentaBDE
			Endocr		18 (reduced serum T4)		
			Bd Wt	72			
23	Mouse (C57BL/6N)	14 d 1x/d (GO)	Hepatic	18	36 (reduced liver vitamin A)		Hallgren et al. 2001 PentaBDE
			Endocr		18 (reduced serum T4)		

Table 3-2. Levels of Significant Exposure to PBDEs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
Immunological/Lymphoreticular							
24	Rat (Sprague-Dawley)	14 d 1x/d (GO)		36			Darnerud and Thuvander 1998 PentaBDE
25	Mouse (C57BL/6N)	14 d 1x/d (GO)		18	36 (reduced in vitro production of IgG in mitogen- stimulated splenocytes)		Darnerud and Thuvander 1998 PentaBDE
26	Mouse (C57BL/6N)	once (GO)		500			Fowles et al. 1994 PentaBDE
27	Mouse (C57BL/6N)	14d 1x/d (GO)		36	72 (reduced antibody response to sheep red blood cells, decreased thymus weight)		Fowles et al. 1994 PentaBDE
Developmental							
28	Rat (Sprague-Dawley)	10 d Gd 6-15 (GO)		100 F		1000 F (subcutaneous edema, delayed skull bone ossification)	Norris et al. 1975; Dow Chemical Co. 1985 77% decaBDE, 22% nonaBDE
29	Rat (CD)	10 d Gd 6-15 (GO)		10	25 (increased resorptions and reduced fetal body weight)		Argus Research Laboratories 1985b OctaBDE

Table 3-2. Levels of Significant Exposure to PBDEs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
30	Rat (CD)	10 d Gd 6-15 (GO)		2.5	10 (minimal increased post-implantation loss)		Life Science Research Israel Ltd. (1987) OctaBDE
31	Rat (CD)	10 d Gd 6-15 (GO)		25	50 (reduced fetal weight and increased skeletal variations associated with maternal tox)		WIL Research Laboratories 1986 OctaBDE
32	Rabbit (New Zealand)	13 d Gd 7-19 (GO)		5 F	15 F (delayed ossification of sternebrae with decreased maternal weight gain)		Breslin et al. 1989 OctaBDE
33	Rat (CD)	10 d Gd 6-15 (GO)		200			Argus Research Laboratories 1985a PentaBDE
34	Rat (Long-Evans)	14 d Gd 6-Gd 20 (GO)		1[b]	10 (reduced serum T4 in fetuses)		Zhou et al. 2002 PBDPE

Table 3-2. Levels of Significant Exposure to PBDEs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	INTERMEDIATE EXPOSURE						
	Systemic						
35	Rat (CD)	28 d (F)	Hepatic	90			IRDC 1976 DecaBDE
			Renal	90			
			Endocr	90			
			Bd Wt	90			
36	Rat (Fischer- 344)	13 wk (F)	Resp	8000			NTP 1986 DecaBDE
			Cardio	8000			
			Gastro	8000			
			Hemato	8000			
			Musc/skel	8000			
			Hepatic	8000			
			Renal	8000			
			Endocr	8000			
			Bd Wt	8000			
37	Mouse (B6C3F1)	13 wk (F)	Resp	9500			NTP 1986 DecaBDE
			Cardio	9500			
			Gastro	9500			
			Hemato	9500			
			Musc/skel	9500			
			Hepatic	9500			
			Renal	9500			
			Endocr	9500			
			Bd Wt	9500			

Table 3-2. Levels of Significant Exposure to PBDEs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
38	Rat (Sprague-Dawley)	30 d (F)	Hemato	800 M			Norris et al., 1973, 1975 77% decaBDE, 22% nonaBDE
			Hepatic	80	800 M (centrilobular cytoplasmic enlargement and vacuolation)		
			Renal	80	800 M (hyaline degenerative cytoplasmic changes)		
			Endocr	8 M	80 M (thyroid hyperplasia)		
			Bd Wt	800			
39	Rat (Sprague-Dawley)	90 d (GO)	Hepatic	2.4 M			Carlson, 1980a OctaBDE
40	Rat (Sprague-Dawley)	90 d (GO)	Hepatic	19.2 M			Carlson, 1980a OctaBDE
41	Rat (CD)	28 d (F)	Hepatic		9 (increased liver weight and enlarged parenchymal cells)		IRDC 1976 OctaBDE
			Renal	90			
			Endocr	90			
			Bd Wt	90			

Table 3-2. Levels of Significant Exposure to PBDEs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
42	Rat (CD)	13 wk (F)	Resp	750 F			IRDC 1977 OctaBDE
			Cardio	750 F			
			Gastro	750 F			
			Hemato	70 F	750 F (reduced erythrocytes, hematocrit and hemoglobin)		
			Hepatic		5 M (cytomegaly with vacuolation and necrosis at higher doses)		
			Renal	50 M	600 M (minimal increase in tubular degenerative changes)		
			Endocr	7 F	50 M (increased thyroid weight with follicular epithelial changes at higher doses)		
			Dermal	750 F			
			Ocular	750 F			
			Bd Wt	70 F	600 M (12% reduced body weight gain)		
43	Rat (Sprague-Dawley)	30 d (F)	Hemato	80 M	800 M (decreased RBC count and packed cell volume)		Norris et al., 1973, 1975 OctaBDE
			Hepatic		8 M (cytoplasmic enlargement and vacuolation)		
			Renal		8 M (hyaline degenerative cytoplasmic changes)		
			Endocr		8 M (thyroid hyperplasia)		
			Bd Wt	800 M			

Table 3-2. Levels of Significant Exposure to PBDEs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
44	Rat (Sprague-Dawley)	90 d (GO)	Hepatic	1.77 M			Carlson 1980a PentaBDE
45	Rat (Sprague-Dawley)	90 d (GO)	Hepatic	14.1 M			Carlson 1980a PentaBDE
46	Rat (CD)	28 d (F)	Hepatic		9 (increased liver weight and enlarged parenchymal cells)		IRDC 1976 PentaBDE
			Renal	90			
			Endocr	90			
			Bd Wt	90			
47	Rat (Sprague-Dawley)	90d (F)	Resp	100			WIL Research Laboratories 1984 PentaBDE
			Cardio	100			
			Gastro	100			
			Hemato	100			
			Musc/skel	100			
			Hepatic		2[c] (minimal LOAEL for hypertrophy, mild degeneration, and slight necrosis)		
			Renal	100			
			Endocr	2	10 (reduced serum T4)		
			Dermal	100			
			Ocular	100			
			Bd Wt	10	100 (reduced weight gain)		

Table 3-2. Levels of Significant Exposure to PBDEs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
48	Rat (Long-Evans)	37 d Gd 6-Pnd 21 (GO)	Endocr	10	30 (reduced maternal serum T4)		Zhou et al. 2002 PentaBDE
	Immunological/Lymphoreticular						
49	Rat (Fischer- 344)	13 wk (F)		8000			NTP 1986 DecaBDE
50	Mouse (B6C3F1)	13 wk (F)		8000			NTP 1986 DecaBDE
51	Rat (CD)	13 wk (F)		750 F			IRDC 1977 OctaBDE
52	Rat (Sprague-Dawley)	90d (F)		100			WIL Research Laboratories 1984 PentaBDE
	Reproductive						
53	Rat (Fischer- 344)	13 wk (F)		8000			NTP 1986 DecaBDE
54	Mouse (B6C3F1)	13 wk (F)		8000			NTP 1986 DecaBDE
55	Rat (Sprague-Dawley)	117 d Pmd 60-Pnd 21 (F)		100			Norris et al. 1975; Dow Chemical Co. 1975 77% decaBDE, 22% nonaBDE

Table 3-2. Levels of Significant Exposure to PBDEs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
56	Rat (CD)	13 wk (F)		600[d] M 750 F			IRDC 1977 OctaBDE
57	Rat (Sprague-Dawley)	90d (F)		100 M 100 F			WIL Research Laboratories 1984 PentaBDE
	Developmental						
58	Rat (Sprague-Dawley)	117 d Pmd 60-Pnd 21 (F)		100			Norris et al. 1975; Dow Chemical Co. 1975 77% decaBDE, 22% nonaBDE
59	Rat (Long-Evans)	37 d Gd 6-Pnd 21 (GO)		1	10 (reduced serum T4 in fetuses and offspring on Gd 20 and Pnd 4 and 14)		Zhou et al. 2002 PBDPE

Table 3-2. Levels of Significant Exposure to PBDEs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	CHRONIC EXPOSURE						
	Systemic						
60	Rat (Fischer- 344)	103 wk (F)	Resp	2550 F			NTP 1986 DecaBDE
			Cardio	2550 F			
			Gastro	1120 M	2240 M (acanthosis of forestomach)		
			Hemato	2550 F			
			Musc/skel	2550 F			
			Hepatic	1120 M	2240 M (degeneration and thrombosis)		
			Renal	2550 F			
			Endocr	2550 F			
			Bd Wt	2550 F			
61	Mouse (B6C3F1)	103 wk (F)	Resp	7780 F			NTP 1986 DecaBDE
			Cardio	7780 F			
			Gastro	3760 F	7780 F (ulcers)		
			Hemato	7780 F			
			Musc/skel	7780 F			
			Hepatic		3200 M (centrilobular hypertrophy and granulomas)		
			Renal	7780 F			
			Endocr		3200 M (follicular cell hyperplasia)		
			Bd Wt	7780 F			

Table 3-2. Levels of Significant Exposure to PBDEs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
62	Rat (Sprague-Dawley)	2 yr (F)	Resp	1.0			Kociba et al. 1975; Norris et al. 1975 77% decaBDE, 22% nonaBDE
			Cardio	1.0			
			Gastro	1.0			
			Hemato	1.0			
			Musc/skel	1.0			
			Hepatic	1.0			
			Renal	1.0			
			Endocr	1.0			
			Ocular	1.0			
			Bd Wt	1.0			
	Immunological/Lymphoreticular						
63	Rat (Fischer- 344)	103 wk (F)			1200[d] F (splenic hematopoiesis)		NTP 1986 DecaBDE
					2240 M (splenic fibrosis and lymphoid hyperplasia)		
64	Mouse (B6C3F1)	103 wk (F)		7780 F			NTP 1986 DecaBDE
65	Rat (Sprague-Dawley)	2 yr (F)		1.0			Kociba et al. 1975; Norris et al. 1975 77% decaBDE, 22% *nona*BDE
	Reproductive						
66	Rat (Fischer- 344)	103 wk (F)		2240[d] M			NTP 1986 DecaBDE
				2550 F			

Table 3-2. Levels of Significant Exposure to PBDEs - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
67	Mouse (B6C3F1)	103 wk (F)		6650[d] M			NTP 1986 DecaBDE
				7780 F			
68	Rat (Sprague-Dawley)	2 yr (F)		1.0 M			Kociba et al. 1975; Norris et al. 1975 77% decaBDE, 22% nonaBDE
				1.0 F			
	Cancer						
69	Rat (Fischer- 344)	103 wk (F)				1120 M (CEL: liver neoplastic nodules)	NTP 1986 DecaBDE
70	Mouse (B6C3F1)	103 wk (F)				3200 M (CEL: hepatocellular adenomas and carcinomas)	NTP 1986 DecaBDE

[a]The number corresponds to entries in Figure 3-2.

[b]Used to derive an acute-duration oral minimal risk level (MRL) of 0.03 mg/kg/day. The MRL was derived by dividing the NOAEL by an uncertainty factor of 30 (10 for extrapolation from animals to humans, 3 for human variability because effects were observed in a sensitive subgroup).

[c]Used to derive an intermediate-duration oral minimal risk level (MRL) of 0.007 mg/kg/day. The MRL was derived by dividing the LOAEL by an uncertainty factor of 300 (3 for converting a minimal LOAEL to a NOAEL, 10 for extrapolation from animals to humans, 10 for human variability).

[d] Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-2. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

BDE = brominated diphenyl ethers; Bd Wt = body weight; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); Endocr = endocrine; (F) = feed; F = Female; (G) = gavage; Gastro = gastrointestinal; gd = gestational day; Gn pig = guinea pig; (GO) = gavage in oil; hemato = hematological; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; pmd = pre-mating day; pnd = post-natal day; occup = occupational; RBC = red blood cell; Resp = respiratory; x = time(s); (W) = drinking water; wk = week(s)

Figure 3-2. Levels of Significant Exposures to PBDEs - Oral

Acute (≤14 days)

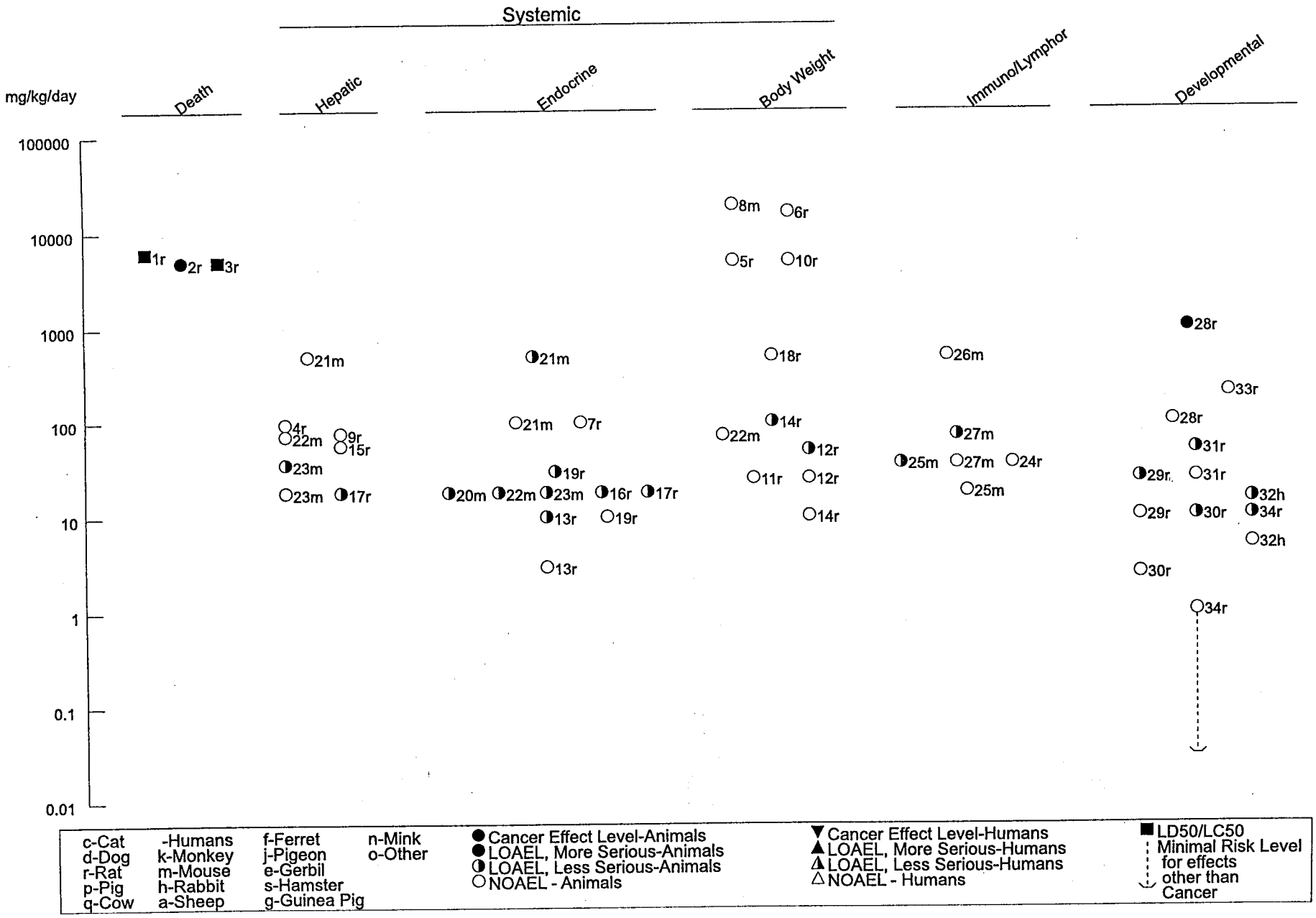


Figure 3-2. Levels of Significant Exposures to PBDEs - Oral (*Continued*)

Intermediate (15-364 days)

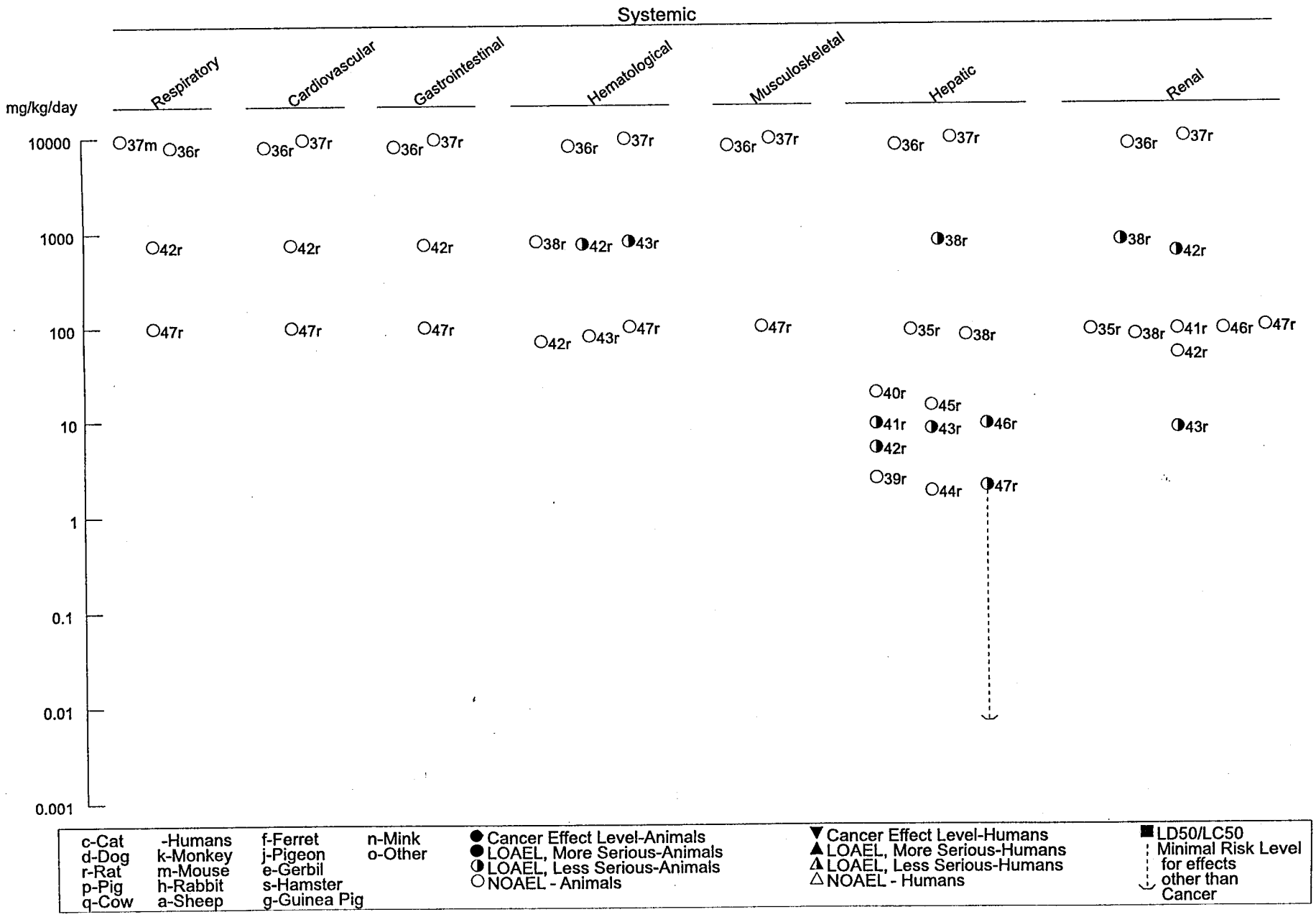


Figure 3-2. Levels of Significant Exposures to PBDEs - Oral (Continued)

Intermediate (15-364 days)

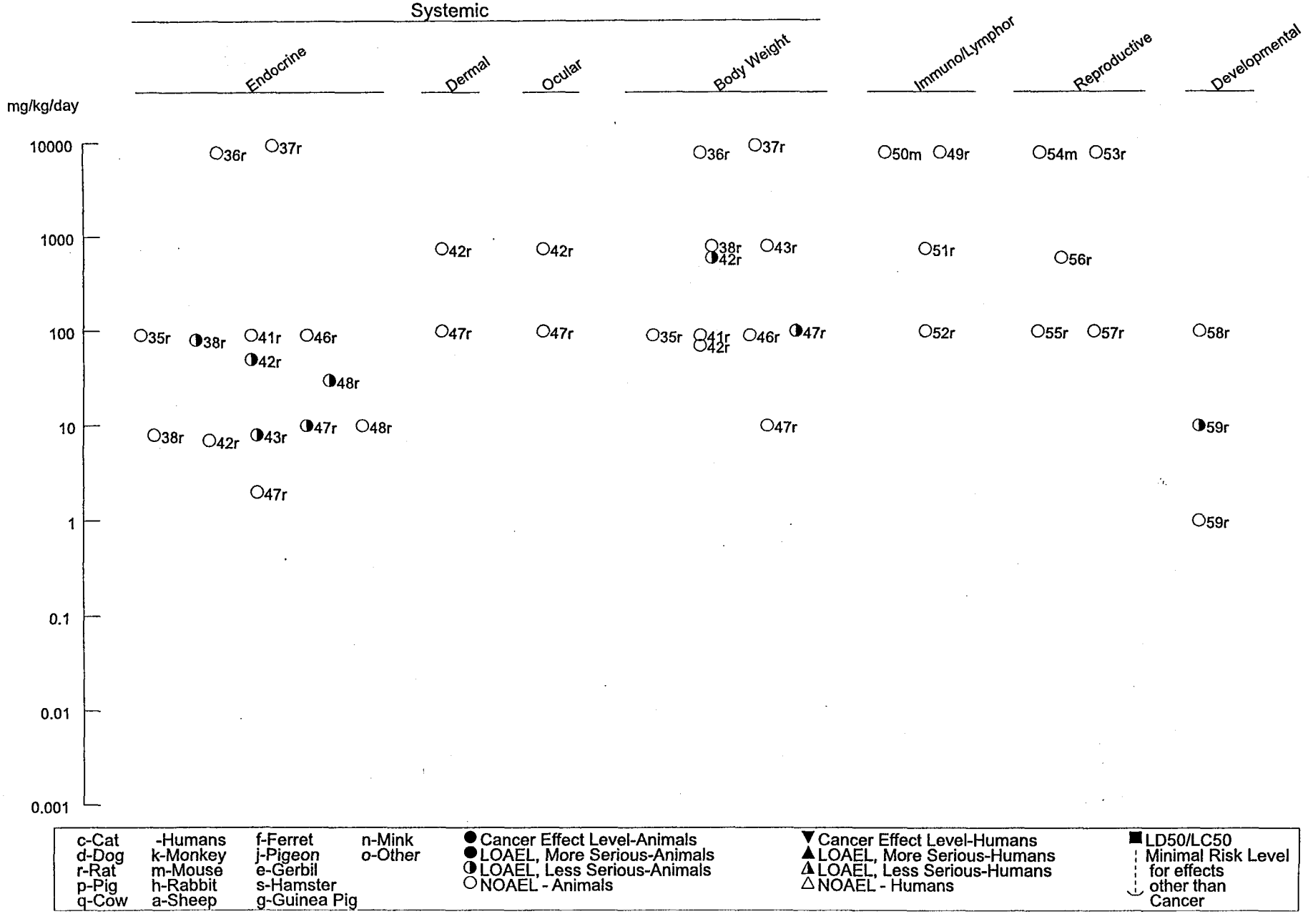
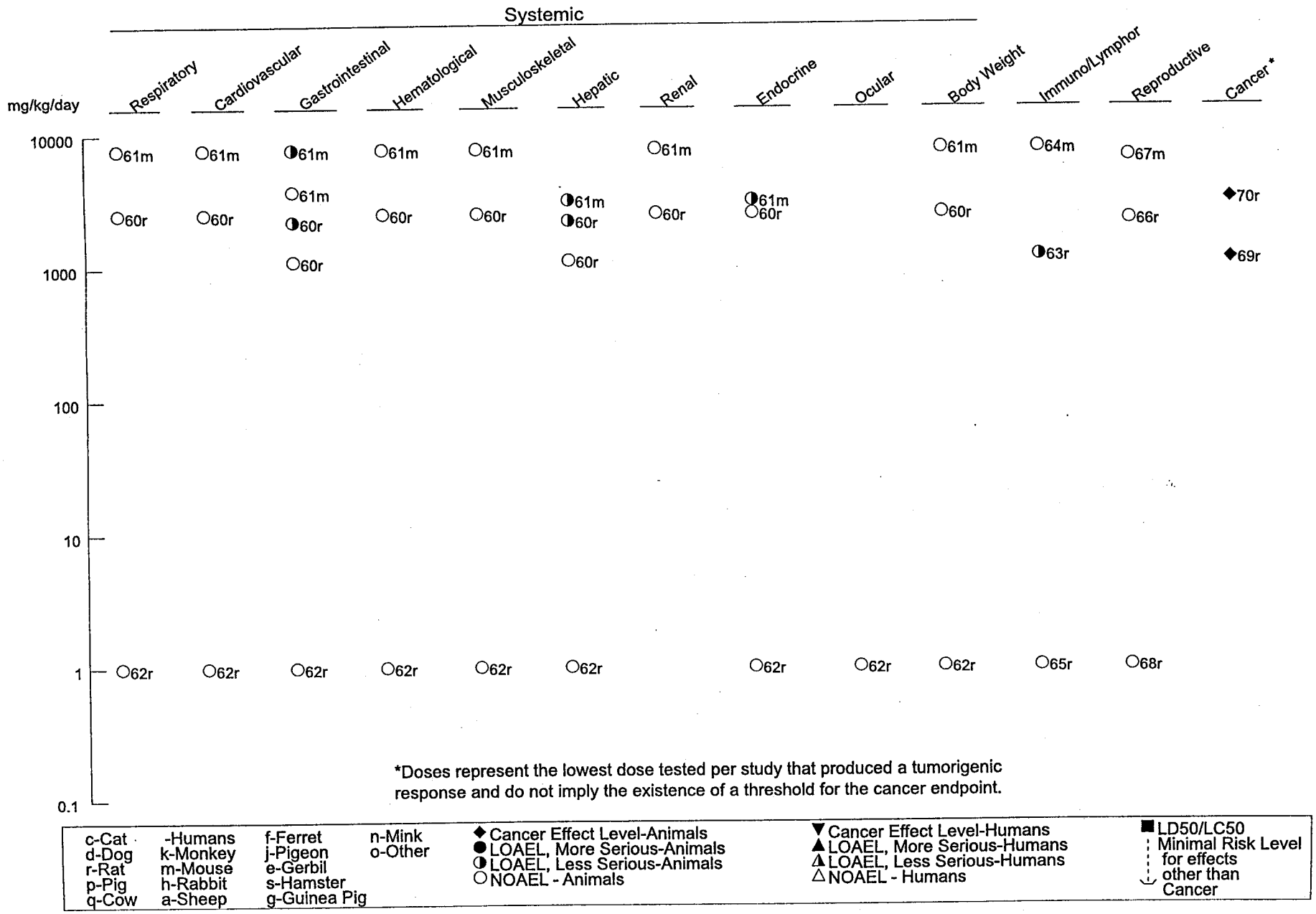


Figure 3-2. Levels of Significant Exposures to PBDEs - Oral (*Continued*)

Chronic (≥365 days)



*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat	-Humans	f-Ferret	n-Mink
d-Dog	k-Monkey	j-Pigeon	o-Other
r-Rat	m-Mouse	e-Gerbil	
p-Pig	h-Rabbit	s-Hamster	
q-Cow	a-Sheep	g-Guinea Pig	

◆ Cancer Effect Level-Animals
● LOAEL, More Serious-Animals
◑ LOAEL, Less Serious-Animals
○ NOAEL - Animals

▼ Cancer Effect Level-Humans
▲ LOAEL, More Serious-Humans
◮ LOAEL, Less Serious-Humans
△ NOAEL - Humans

■ LD50/LC50
⋮ Minimal Risk Level for effects other than Cancer

exposure to #10 mg/kg/day for 25 weeks (NTP 1983) or #30 mg/kg/day for 30 days (Gupta et al. 1981), or dietary exposure to #3.9 mg/kg/day for up to 105 weeks (NTP 1992). Guinea pig lung histology was unaffected by exposures of #20 mg/kg/day of an unspecified PBB mixture in the feed for 30 days (Sleight and Sanger 1976). Relative lung weights increased in mink that died following exposure to #2.4 mg/kg/day FireMaster FF-1 for 313 days, but it is unclear if this effect is adverse because the animals had lost weight and histopathology was not reported (Aulerich and Ringer 1979; Ringer et al. 1981). Effects in six pregnant cows given 67 mg/kg/day of FireMaster BP-6 in capsules for up to 60 days included increased respiratory rate and occasional nasal discharge (Moorhead et al. 1977). Necropsy following sacrifice between days 33 and 66 because of impending death due to poor health showed gross pneumonia (one cow), microscopic lesions of early purulent bronchopneumonia (two cows), and petechial hemorrhages of the tracheal mucosa (one cow). No histological changes were observed in the trachea or lungs treated with #0.65 mg/kg/day and observed for 1–140 days following the end of treatment. Information on respiratory effects of octabromobiphenyl mixture or other PBB mixtures in animals was not located.

Polybrominated Diphenyl Ethers. No studies were located regarding respiratory effects in humans after oral exposure to PBDEs.

Effects of PBDEs on respiratory function have not been studied in orally-exposed animals. No histopathological changes in respiratory tract tissues were found in rats and mice fed decaBDE in estimated doses of #8,000 and #9,500 mg/kg/day, respectively, for 13 weeks (NTP 1986). In chronic dietary studies, there was no respiratory tract histopathology in rats that were fed #1.0 mg/kg/day of a 77.4% decaBDE mixture (containing 21.8% nonaBDE and 0.8% octaDBDE) for 2 years (Kociba et al. 1975; Norris et al. 1975b), or in rats and mice exposed to decaBDE at estimated doses of #2,550 and #7,780 mg/kg/day, respectively, for 103 weeks (NTP 1986).

No histopathological changes in the respiratory tract were found in dietary studies of rats exposed to #750 mg/kg/day of octaBDE for 13 weeks (IRDC 1977) or #100 mg/kg/day of pentaBDE for 90 days (WIL Research Laboratories 1984).

Cardiovascular Effects.

Polybrominated Biphenyls. No studies were located regarding cardiovascular effects in humans after oral exposure to PBBs.

Animal data do not generally indicate cardiovascular toxicity of PBBs even at lethal doses, but cardiovascular function was not evaluated in most studies. No exposure-related histological changes in the heart were observed in rats administered FireMaster FF-1 in a single dose of 200 or #1,000 mg/kg and observed for 2–23 months posttreatment (Kimbrough et al. 1978a, 1981), or #30 mg/kg/day for 2 weeks (Gupta et al. 1981; Kimbrough et al. 1978b, 1981). Mice exposed to #30 mg/kg/day FireMaster FF-1 for 2 weeks also showed no histological alterations in the heart (Gupta et al. 1981). In intermediate-and chronic-duration studies with rats, FireMaster FF-1 or FireMaster BP-6 dosages of #30 mg/kg/day by gavage for 30 days (Gupta et al. 1981), #10 mg/kg/day in the diet for 30 days (Akoso et al. 1982a; Sleight et al. 1978), #10 mg/kg/day by gavage for 25 weeks (NTP 1983), or #1.5 mg/kg/day in the diet for up to 104 weeks (NTP 1992) did not alter heart weight or histology. Rat heart histology also was unaffected by exposure to 50 mg/kg/day of an unspecified PBB mixture for 30 days (Sleight and Sanger 1976). Rats exposed to #5 mg/kg/day FireMaster BP-6 for 30 days exhibited no exposure-related changes in blood pressure, but histology or other cardiovascular end points were not evaluated (McCormack et al. 1978). Rats exposed to octabromobiphenyl mixture in dosages of #1 mg/kg/day for 8 months or #800 mg/kg/day for 30 days showed no changes in heart weight, but histology or function was not evaluated (Norris et al. 1975a). In studies with mice, FireMaster FF-1 produced no changes in heart weight or histology following gavage exposure to #10 mg/kg/day for 25 days (Gupta et al. 1981), #30 mg/kg/day for 30 days (NTP 1983), or dietary exposure to #3.9 mg/kg/day for up to 105 weeks (Chhabra et al. 1993; NTP 1992). No effects on heart relative weight or histology were reported in mink that died following exposure to #2.4 mg/kg/day FireMaster FF-1 for 313 days (Aulerich and Ringer 1979; Ringer et al. 1981). Relative heart weights were increased in swine exposed to #8 mg/kg/day of an unspecified PBB mixture for 16 weeks, but gross pathology was normal and histology was not evaluated (Ku et al. 1978). Necropsy of a monkey that died following ingestion of 0.73 mg/kg/day FireMaster FF-1 for 25 weeks showed an enlarged heart, but histology was not evaluated and a similar effect was not reported in two other monkeys exposed to higher dosages (Allen et al. 1978; Lambrecht et al. 1978). Mean heart rate was 32% lower than the pre-exposure value in pregnant cows that were treated with 67 mg/kg/day of FireMaster BP-6 in capsules for 10 days (Moorhead et al. 1977). Necropsy following sacrifice between days 33 and 66 because of impending death due to poor health showed petechial and ecchymotic hemorrhages of the myocardium and endocardium in two of six cows. No cardiovascular effects were observed in cows given #0.65 mg/kg/day and observed for 1–140 days following the end of treatment.

Polybrominated Diphenyl Ethers. No studies were located regarding cardiovascular effects in humans after oral exposure to PBDEs.

Effects of PBDEs on cardiovascular function have not been studied in orally exposed animals. No histopathological changes in the heart were found in rats and mice fed decaBDE in estimated doses of #8,000 and #9,500 mg/kg/day, respectively, for 13 weeks (NTP 1986). In chronic dietary studies, there was no cardiac histopathology in rats that were fed #1.0 mg/kg/day of a 77.4% decaBDE mixture (containing 21.8% nonaBDE and 0.8% octaDBDE) for 2 years (Norris et al. 1975b), or in rats and mice exposed to decaBDE in estimated doses of #2,550 and #7,780 mg/kg/day, respectively, for 103 weeks (NTP 1986).

No histopathological changes in the heart were found in dietary studies of rats exposed to #750 mg/kg/day of octaBDE for 13 weeks (IRDC 1977) or #100 mg/kg/day of pentaBDE for 90 days (WIL Research Laboratories 1984).

Gastrointestinal Effects.

Polybrominated Biphenyls. No symptoms of gastrointestinal effects were reported by residents of quarantined Michigan farms in an epidemiological study conducted by the U.S. Center for Disease Control and the Michigan Department of Public Health (Landrigan et al. 1979). In a medical history survey conducted by the Environmental Science Laboratory of the Mount Sinai School of Medicine, no statistically significant difference was observed between the prevalence rates of gastrointestinal symptoms for 933 Michigan residents who were likely to have ingested PBB-contaminated food and the rates for a control group of 229 Wisconsin farm residents (Anderson et al. 1978c). The Michigan residents were examined .3 years after the contamination episode occurred. No other studies were located regarding gastrointestinal effects in humans after oral exposure to PBBs.

Gastric lesions have developed in various animals that ingested PBBs, particularly after prolonged exposure to FireMaster FF-1 or FireMaster BP-6. No exposure-related histological changes in the gastrointestinal tract or esophagus were observed in rats administered FireMaster FF-1 in a single dose #1,000 mg/kg and observed for 2–23 months posttreatment (Kimbrough et al. 1978b, 1981). Rats or mice exposed to #30 mg/kg/day FireMaster FF-1 for 2 weeks also showed no histological alterations in the gastrointestinal tract (esophagus not examined) (Gupta et al. 1981). In intermediate-duration studies, the gastrointestinal tract of rats exposed to FireMaster BP-6 or FireMaster FF-1 by gavage or diet at #50 mg/kg/day for 4–4.5 weeks showed no histopathological changes (esophagus not examined) (Akoso et al. 1982a; Gupta and Moore 1979; Gupta et al. 1981; Sleight and Sanger 1976; Sleight et al. 1978). Histological examination of the gastrointestinal tract of rats administered FireMaster FF-1 by gavage for

25 weeks showed significantly increased incidences of gastric ulcers at $1 mg/kg/day and hyperplastic gastropathy at $3 mg/kg/day after lifetime observation (23 months). These gastric effects were not observed in rats examined at the end of the gavage treatment period, although similar changes (forestomach hyperplasia, inflammation, and ulceration) occurred in rats exposed to 1.5 mg/kg/day FireMaster FF-1 in the diet for up to 104 weeks (Chhabra et al. 1993; NTP 1992). In the only study of a decabromobiphenyl mixture, rats were fed estimated dosages as high as 100 mg/kg/day for 13 weeks (Millischer et al. 1980). A comprehensive histology evaluation was performed in this study, but the liver is the only tissue specifically mentioned as having been examined. Due to the total number of tissues examined (21) and route of exposure, it is probable that the gastrointestinal tract was examined but not discussed because no histopathologic changes were observed.

Gastrointestinal tract histology was normal in mice exposed to FireMaster FF-1 dosages of #10 mg/kg/day by gavage for 25 weeks or #3.9 mg/kg/day in the diet for up to 105 weeks (NTP 1983, 1992). FireMaster FF-1 produced no histological changes in the gastrointestinal tract of mice exposed to #30 mg/kg/day for 30 days (Gupta et al. 1981). Gross pathologic examination of swine administered an unspecified PBB mixture for 16 weeks showed that the glandular portion of the stomach "appeared somewhat hyperplastic" (additional details were not reported, and histology was not evaluated) at 8 mg/kg/day, but not at 1 mg/kg/day (Ku et al. 1978). Biopsies of two monkeys performed following their ingestion of 0.73 or 1.43 mg/kg/day FireMaster FF-1 for 12 weeks showed proliferation of gastric mucosal cells, focal areas of infiltration of chronic inflammatory cells, and isolated penetrations of the gastric mucosa into the underlying submucosa (Allen et al. 1978; Lambrecht et al. 1978). Necropsies performed after 25 or 50 weeks of exposure also showed hyperplastic gastroenteritis and, in the low-dose monkey (that died of "severe gastrointestinal changes"), severe ulcerative colitis. Hyperplastic gastroenteritis was described in another monkey exposed to a higher dosage (18 mg/kg/day) of FireMaster FF-1 for 137 days (Allen et al. 1978). Gastrointestinal effects in six pregnant cows that were administered 67 mg/kg/day of FireMaster BP-6 in capsules for up to 60 days included diarrhea, dehydration (possibly a result of the diarrhea), and occasional constipation (Moorhead et al. 1977). Necropsy following sacrifice between days 33 and 66 because of impending death due to poor health showed edema and hemorrhage of the colon and rectum mucosa, although histology was normal in the esophagus, rumen, omasum, and reticulum. No histological changes were observed in the gastrointestinal tract of cows with #0.65 mg/kg/day and observed 1 or 140 days following the end of treatment.

Polybrominated Diphenyl Ethers. No studies were located regarding gastrointestinal effects in humans after oral exposure to PBDEs.

No histopathological changes in gastrointestinal tract tissues were found in rats and mice fed decaBDE in estimated doses of #8,000 and #9,500 mg/kg/day, respectively, for 13 weeks (NTP 1986). In chronic dietary studies, there was no gastrointestinal tract histopathology in rats that were fed #1.0 mg/kg/day of a 77.4% decaBDE mixture (containing 21.8% nonaBDE and 0.8% octaDBDE) for 2 years (Kociba et al. 1975; Norris et al. 1975b). Higher dietary doses of decaBDE for 103 weeks caused acanthosis of the forestomach in rats exposed to 2,240 mg/kg/day (no effects at #1,200 mg/kg/day) and stomach ulcers in mice exposed to 7,780 mg/kg/day (no effects at #3,760 mg/kg/day) (NTP 1986).

No histopathological changes in the gastrointestinal tract were found in dietary studies of rats exposed to #750 mg/kg/day of octaBDE for 13 weeks (IRDC 1977) or #100 mg/kg/day of pentaBDE for 90 days (WIL Research Laboratories 1984).

Hematological Effects.

Polybrominated Biphenyls. No studies were located regarding hematological effects in humans after oral exposure to PBBs.

In animals, hematologic changes indicative of possible anemia are common findings in animals resulting from longer-term exposure to PBBs. Comprehensive hematological examinations in rats and mice administered #30 mg/kg/day FireMaster FF-1 for 2 weeks showed no exposure-related changes (Gupta et al. 1981). No additional information on hematology in animals following acute-duration exposure to PBBs was located. In intermediate-duration studies, no consistent hematological changes were found in rats exposed to #10 mg/kg/day FireMaster BP-6 for 30 days (Akoso et al. 1982a; Sleight et al. 1978). Some hematologic effects occurred in rats at higher dosages or longer durations. Exposure to 30 mg/kg/day FireMaster FF-1 for 4.5 weeks significantly reduced hemoglobin concentration, packed cell volume (PCV), and platelet count in rats evaluated up to . 60 days postexposure (Gupta and Moore 1979). In another study in which rats were administered the same dosages of FireMaster FF-1 (#30 mg/kg/day) for 30 days, longer postexposure (up to 90 days) evaluation revealed transient responses (Gupta et al. 1981). Transitory and slight but significant ($p<0.05$) decreases in red blood cell count, hemoglobin concentration, and PCV values were found; they returned to control levels by 60-days post-dosing. No consistent hematological changes were observed in rats administered #50 mg/kg/day of an unspecified PBB mixture for 30 days (Sleight and Sanger 1976) or #10 mg/kg/day FireMaster FF-1 for 6 months (Luster et al. 1980). Rats exposed to FireMaster FF-1 for 25 weeks showed no hematological changes at 0.1 mg/kg/day, but had dose-related, significantly decreased hemoglobin, mean corpuscular hemoglobin

(MCH), mean corpuscular volume (MCV), and PCV at $0.3 mg/kg/day, and increased total leukocytes at $1 mg/kg/day; there were no effects on erythrocyte or platelet counts (NTP 1983). Mice similarly treated for 25 weeks had decreased erythrocyte count and MCV at $0.3 mg/kg/day and decreased platelets and lymphocytes at $1 mg/kg/day, but no hematological effects were noted at 0.1 mg/kg/day (NTP 1983). No hematologic alterations were found in mice exposed to FireMaster FF-1 at dosages of #10 mg/kg/day for 6 months or #30 mg/kg/day for 30 days (Gupta et al. 1981; Luster et al. 1980).

Hematologic evaluation of swine treated with an unspecified PBB mixture for 16 weeks showed significantly decreased hemoglobin and hematocrit values in two of four animals exposed to 8 mg/kg/day at week 6, after which values returned to normal or near-normal within 2 weeks (Ku et al. 1978). Decreased PCV and serum protein developed in monkeys exposed to FireMaster FF-1 in dosages of $0.73 mg/kg/day for $25 weeks (two animals); additional hematologic effects observed in one monkey exposed to 18 mg/kg/day for 137 days were decreased erythrocyte and white blood cell counts (Allen et al. 1978; Lambrecht et al. 1978). No hematological changes were measured in cows treated with #0.65 mg/kg/day FireMaster BP-6 in capsules for 60 days, and observed for up to 140 days following the end of treatment (Moorhead et al. 1977). Similar treatment with 67 mg/kg/day did not cause abnormal hematologic indices in four of six cows; changes in the other two animals (e.g., leukocytosis, increased PCV) have uncertain toxicologic significance because the animals at this dose were sacrificed between days 33 and 66 because of impending death due to poor health.

Studies of hematologic effects of octabromobiphenyl mixture, performed only in rats, showed significantly decreased red blood cell count and PCV following 800 mg/kg/day for 30 days, but no hematological changes resulting from #1 mg/kg/day for 8 months (Norris et al. 1975a). In the only study of a decabromobiphenyl mixture, dietary administration of 100 mg/kg/day for 13 weeks caused no hematologic changes in rats (Millischer et al. 1980). Erythrocyte and leucocyte counts, differential leukocyte count, and hematocrit and hemoglobin levels were measured.

Polybrominated Diphenyl Ethers. No studies were located regarding hematological effects in humans after oral exposure to PBDEs.

OctaBDE caused red blood cell effects in intermediate-duration dietary studies in rats. Hematological changes included decreases in red blood cell count and packed cell volume following exposure to 800 mg/kg/day for 30 days (no effects at #80 mg/kg/day) (Norris et al. 1973, 1975a, 1975b) and reduced

erythrocytes, hematocrit, and hemoglobin following exposure to 750 mg/kg/day for 13 weeks (no effects at #70 mg/kg/day) (IRDC 1977).

DecaBDE and pentaBDE did not induce hematological effects in animals. In dietary studies with decaBDE, no hematological changes were found in rats exposed to #800 mg/kg/day for 30 days (Norris et al. 1973, 1975a, 1975b), #8,000 mg/kg/day for 13 weeks (NTP 1986), or #2,550 mg/kg/day for 103 weeks (NTP 1986), or in mice exposed to #9,500 mg/kg/day for 13 weeks or #7,780 mg/kg/day for 103 weeks (NTP 1986). There also were no hematological effects in rats exposed by diet to #1.0 mg/kg/day of a 77.4% decaBDE mixture (containing 21.8% nonaBDE and 0.8% octaDBE) for 2 years (Kociba et al. 1975; Norris et al. 1975b) or #100 mg/kg/day of pentaBDE for 90 days (WIL Research Laboratories 1984).

Musculoskeletal Effects.

Polybrominated Biphenyls. Symptoms of musculoskeletal effects, described as "joint pain" and "swelling in joints," were frequently cited health complaints in two epidemiological studies of groups of Michigan residents who were likely to have ingested PBB-contaminated food (Anderson et al. 1978c; Landrigan et al. 1979). Although one study demonstrated a statistically significant difference between the prevalence rate for these types of symptoms in Michigan residents compared with nonexposed residents of Wisconsin farms (Anderson et al. 1978c), neither study demonstrated a positive association between serum PBB levels and the prevalence rates for symptoms of musculoskeletal effects.

There are no pathology data indicating that PBBs produce effects in musculoskeletal tissues of animals. No exposure-related histological changes in muscle or bone marrow were observed in rats that were administered a single 1,000 mg/kg dose of FireMaster FF-1 and observed for 2 years (Kimbrough et al. 1981). Rats and mice exposed to #30 mg/kg/day FireMaster FF-1 for 2 weeks showed no histological alterations in muscle or sternum (Gupta et al. 1981). In intermediate-and chronic-duration studies, rats and/or mice exposed to FireMaster FF-1 or FireMaster BP-6 dosages of #30 mg/kg/day by gavage for 30 days (Gupta et al. 1981), #10 mg/kg/day in the diet for 30 days (Akoso et al. 1982a; Sleight et al. 1978), #10 mg/kg/day by gavage for 25 weeks (NTP 1983), or #3.9 mg/kg/day in the diet for up to 105 weeks (NTP 1992) showed no histopathological changes in muscle or bone. A dosage of an unspecified PBB mixture as high as 50 mg/kg/day for 30 days produced no histopathological changes in rat muscle (Sleight and Sanger 1976). Excess porphyrins were detected in bone and/or teeth by fluorescence under ultraviolet light in some of the rat studies (Gupta and Moore 1979; NTP 1983), but

this appears to be a consequence of altered porphyrin metabolism (Hill 1985). No histological alterations were observed in sternebrae bone marrow of pregnant cows given FireMaster BP-6 in capsules for up to 60 days (Moorhead et al. 1977). Cows treated with 67 mg/kg/day were necropsied following sacrifice between days 33 and 66 because of impending death due to poor health, and those treated with nonlethal lower dosages of #0.65 mg/kg/day were examined 1 or 140 days following the end of treatment.

Polybrominated Diphenyl Ethers. No studies were located regarding musculoskeletal effects in humans after oral exposure to PBDEs.

Dietary studies with decaBDE found no histopathological changes in musculoskeletal tissues in rats exposed to #8,000 mg/kg/day for 13 weeks (NTP 1986), #1.0 mg/kg/day (77.4% containing 21.8% nonaBDE and 0.8% octaDBE) for 2 years (Kociba et al. 1975; Norris et al. 1975b), or #2,550 mg/kg/day for 103 weeks (NTP 1986), or in mice exposed to #9,500 mg/kg/day for 13 weeks or #7,780 mg/kg/day for 103 weeks (NTP 1986). A study of pentaBDE found no musculoskeletal changes in rats exposed dietary doses of #100 mg/kg/day for 90 days (WIL Research Laboratories 1984). No information was located on possible musculoskeletal effects of octaBDE.

Hepatic Effects.

Polybrominated Biphenyls. Results from several studies of humans exposed to PBBs do not demonstrate, in general, a conclusive association between adverse effects on the liver and oral exposure to PBBs. In a study in which serum was collected in 1974, 1977, 1978, and 1979 from 89, 240, 220, and 200 individuals, respectively, who were predominately residents of quarantined Michigan farms, no consistent statistically significant correlations were found between serum PBB levels and levels of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) or serum bilirubin (Kreiss et al. 1982). The prevalence rates of Michigan residents with abnormally high levels of SGPT (12.7% prevalence rate), SGOT (12.7%), or lactate dehydrogenase (8.6%) were statistically significantly higher than comparable rates for residents of Wisconsin farms (2.7, 2.0, and 3.3%) (Anderson et al. 1979). A contingency table analysis indicated that the prevalence of abnormal SGPT values in Michigan residents with serum PBB levels #1 ppb (8%) was lower than the prevalence rate for residents with serum PBB levels $1 ppb (14%), but correlation coefficients for serum PBB levels and serum liver enzyme levels were uniformly low ($r<0.1$) (Anderson et al. 1979). Physical examinations of Michigan residents (37 men and 9 women) with known exposure to PBBs and a history of incapacitating health care complaints revealed that 72% of the subjects displayed mildly enlarged livers, which were

associated with elevations in serum liver enzymes (SGOT and SGPT) predominately less than 2-fold above normal values (Stross et al. 1979). Mildly enlarged livers, confirmed by liver scanning, were observed only in 17 and 7% of 23 Michigan residents with known PBB exposure and incapacitating health complaints and 28 workers involved in the manufacture and distribution of PBBs, respectively; however, these subjects had histories of either substantial alcohol intake or exposure to multiple chemicals (Stross et al. 1981). Results of a caffeine breath test, discussed in Section 3.8.2, suggest that PBBs may have induced hepatic microsomal enzymes in exposed Michigan residents (Lambert et al. 1990).

Hepatic effects of PBBs are documented in various animal species although rats have been the species tested most extensively. The changes appear to be similar among species and reversible when mild. Characteristic hepatic effects include proliferation of the smooth endoplasmic reticulum, microsomal enzyme induction, increased serum levels of liver-associated enzymes indicative of possible hepatocellular damage, liver enlargement, hepatocyte vacuolation and fat deposition, fibrosis, and necrosis. PBBs also cause alterations in levels of cholesterol and other lipids in liver and serum, levels of vitamin A in liver and urine, and levels of porphyrins in liver, other tissues, and urine. These changes could be secondary to liver damage or due to direct effects on lipid, vitamin A, and porphyrin metabolism, which occurs primarily in the liver. Induction of microsomal enzymes by PBBs is a sensitive effect generally regarded as an adaptive response of the liver rather than as a manifestation of hepatotoxicity *per se* (Guzelian 1985). Although not necessarily adverse, induction of microsomal enzymes could alter the rate or pathways of metabolism of other xenobiotic or endogenous substances and increase activation of promutagens and procarcinogens or increase detoxification pathways. In addition, the induction of some microsomal enzyme activities is an indicator of exposure to PBBs and related compounds (Ah receptor agonists), which elicit a well known pattern of toxic responses (see Chapter 2). PBB-related liver enlargement is usually associated with hepatocyte enlargement and an increase in smooth endoplasmic reticulum and/or increased microsomal enzymatic activity; therefore, it is not considered an adverse effect unless accompanied by other biochemical changes and/or histological alterations.

Rats administered FireMaster FF-1 in a single 1,000 mg/kg dose and observed for 2–23 months posttreatment or a lethal dose of 1,000 mg/kg/day for 2 weeks developed enlarged livers with fatty and necrotic changes leading to fibrosis (Gupta and Moore 1979; Kimbrough et al. 1978b, 1981). Lower single doses of FireMaster FF-1 caused vacuolation and some biochemical changes (e.g., increased serum cholesterol and phospholipids, decreased serum retinol) at 500 mg/kg (Bernert et al. 1983; Kimbrough et

al. 1980), and hepatic porphyrin accumulation with no histologic changes at 200 mg/kg (Kimbrough et al. 1981). Repeated exposure to lower dosages of $3 mg/kg/day FireMaster FF-1 for 2 weeks (Gupta et al. 1981) or 5 mg/kg/day FireMaster BP-6 for 10 days (Raber and Carter 1986) caused hepatocyte enlargement and some fatty and single-cell necrotic changes in weanling and young rats. A limited amount of data suggest that octabromobiphenyl mixture-induced hepatic effects in rats are milder than for FireMaster mixtures at similar dosages. Fatty changes appear to be the most severe hepatic histopathologic effect of octabromobiphenyl observed following a single 1,000 mg/kg dose or doses of 3,000 mg/kg/day for 2 days and 6.53 mg/kg/day (but not 0.66 mg/kg/day) for 2 weeks (Lee et al. 1975a, 1975b; Waritz et al. 1977). In studies with mice, a single dose of 36 mg/kg FireMaster BP-6 increased liver weight (histology not evaluated) and had no consistent effects on disposition of injected ouabain or indocyanine green, indicating that hepatic function was not compromised (Cagen et al. 1977). Sporadic increases in the clearance of ouabain and indocyanine green were attributed to increased liver size. Exposure to 130 mg/kg/day FireMaster BP-6, for 11 days caused focal areas of coagulative necrosis (Corbett et al. 1975) and $3 mg/kg/day FireMaster FF-1 for 2 weeks caused scattered necrosis in mice (Gupta et al. 1981).

In intermediate-duration studies with rats, dosages $0.05 mg/kg/day FireMaster BP-6 for 20 days induced hepatic microsomal enzymes but histology was not evaluated (Babish et al. 1978). Dose-related hepatocyte swelling and vacuolation were induced by $0.1 mg/kg/day FireMaster BP-6 for 30 days (Akoso et al. 1982a), lipid accumulation, porphyrin levels, and atypical foci were increased by $0.3 mg/kg/day FireMaster FF-1 for 25 weeks (lethal dose) (NTP 1983), and bile duct hyperplasia was induced by 0.5 mg/kg/day FireMaster BP-6 for 82 days (Darjono et al. 1983). Rats exposed to higher, but not necessarily lethal, dosages of FireMaster FF-1 or FireMaster BP-6 for 1–3 months showed progression of these effects, including marked degenerative changes and porphyrin accumulation in these and other studies (Gupta and Moore 1979; Gupta et al. 1981; Kasza et al. 1978a; McCormack et al. 1978; Sleight and Sanger 1976; Sleight et al. 1978). In the only chronic study, incidences of hepatocellular hypertrophy, cytoplasmic vacuolation, atypical foci, and oval cell hyperplasia were increased in rats fed $0.5 mg/kg/day FireMaster FF-1 for up to 104 weeks (Chhabra et al. 1993; NTP 1992). Compared to this adult-only exposure, combined perinatal and adult exposure resulted in increased incidences of oval cell hyperplasia at 0.5 mg/kg/day and hypertrophy, cytoplasmic vacuolation, and bile duct fibrosis at 1.5 mg/kg/day. Studies of octabromobiphenyl mixture in rats have shown hepatic effects (e.g., hypertrophy and hyperplasia of centrilobular cells, vacuolation, and other fatty degenerative changes) at dosages $6.53 mg/kg/day for 4 weeks (Lee et al. 1975b; Norris et al. 1975a; Waritz et al. 1977), but normal liver histology at #1 mg/kg/day for 8 months (Norris et al. 1975a). A 13-week dietary study with

decabromobiphenyl mixture found that hepatic effects in rats, including vacuolization and distension of centrilobular hepatocytes often accompanied by slightly increased lipid, did not occur at dosages <100 mg/kg/day (Millischer et al. 1980). Information on hepatic effects of octabromobiphenyl mixture and decabromobiphenyl in species other than the rat was not located.

In mice, exposure to FireMaster FF-1 for 25 weeks increased liver weight, porphyrin content, and SGOT at $0.3 mg/kg/day and hepatocyte swelling occurred at $1 mg/kg/day (NTP 1983). Hepatic effects in mice exposed to $1.3 mg/kg/day FireMaster FF-1 for up to 105 weeks included hepatocyte hypertrophy, vacuolization, and necrosis; bile duct hyperplasia also developed (NTP 1992). Dosages $3 mg/kg/day for 4–6 weeks, but not 0.3 mg/kg/day, also induced hepatocyte necrosis and/or vacuolation in mice (Gupta et al. 1981; Loose et al. 1981; NTP 1983). Fatty changes and centrilobular necrosis developed in pregnant swine fed $1.25 mg/kg/day, but not 0.125 mg/kg/day, FireMaster BP-6 for 12 weeks during the second half of gestation through lactation (Werner and Sleight 1981). This adverse effect level cannot be corroborated in nonpregnant swine exposed to #8 mg/kg/day of unspecified PBBs for 16 weeks due to lack of liver histology evaluations, although relative liver weight increased at $1 mg/kg/day and no gross changes were observed (Ku et al. 1978). Guinea pigs appear to be particularly susceptible to hepatic effects of PBBs (unspecified) as indicated by ultrastructural vacuolation and formation of myelin bodies in hepatocytes following exposure to $0.04 mg/kg/day for 30 days; liver weights were increased at 0.4 mg/kg/day and histological vacuolation and severe centrilobular fatty change were observed at a lethal dose of 4 mg/kg/day (Sleight and Sanger 1976). Mink that ingested $0.24 mg/kg/day FireMaster FF-1 for #313 days showed increased liver weight and fatty infiltration (Aulerich and Ringer 1979; Ringer et al. 1981). In monkeys, lethal FireMaster FF-1 dosages $0.73 mg/kg/day for 25–50 weeks caused hepatocyte enlargement with increased lipid droplets, bile duct hyperplasia, increased SGPT, and decreased serum cholesterol (Allen et al. 1978; Lambrecht et al. 1978). Effects in six pregnant cows given 67 mg/kg/day of FireMaster BP-6 in capsules for up to 60 days included increased serum lactic dehydrogenase (LDH) and SGOT (Moorhead et al. 1977). Necropsy following sacrifice between days 33 and 66 because of impending death due to poor health showed increased liver weight and pathologic liver changes including friable appearance, glycogen depletion in hepatocytes, sinusoidal dilation, and scattered areas of early fatty degeneration. In general, the hepatic effects observed in cows are less pronounced than in other species at lethal doses. No adverse hepatic effects were observed in cows treated with #0.65 mg/kg/day and examined 1 or 140 days following the end of treatment.

Polybrominated Diphenyl Ethers. No studies were located regarding hepatic effects in humans after oral exposure to PBDEs.

No information was located on adverse hepatic effects of acute-duration oral exposure to PBDEs in animals. Intermediate- and chronic-duration studies in rodents indicate that the liver is a target of toxicity of PBDEs. Repeated dietary exposure to PBDEs typically caused liver enlargement with or without degenerative changes, and effects were generally dose-related in incidence and severity, more frequent and pronounced in males than females, and more severe with octaBDE and pentaBDE than decaBDE. Hepatic effects induced by DBDPO included increased liver weights at $80 mg/kg/day with centrilobular cytoplasmic enlargement and vacuolation at 800 mg/kg/day in male rats exposed for 30 days (Norris et al. 1973, 1975a, 1975b), degeneration and thrombosis in male rats exposed to 2,240 mg/kg/day for 103 weeks (NTP 1986), and centrilobular hypertrophy and granulomas in male mice exposed to $3,200 mg/kg/day for 103 weeks (NTP 1986). OctaBDE caused increased liver weight and histopathological changes such as hepatocellular enlargement and vacuolation in rats exposed to doses as low as 5–8 mg/kg/day (lowest tested doses) for 4–13 weeks (IRDC 1976, 1977; Norris et al. 1973, 1975a, 1975b). Hepatic effects in rats exposed to octaBDE for 13 weeks included increases in absolute and/or relative liver weight at $5-7 mg/kg/day and liver lesions in 40% of males at 5 mg/kg/day and 100% of both sexes at $50 mg/kg/day (IRDC 1977). The lesions were dose-related in severity as well as incidence and characterized by cytomegaly, change in hepatocytic cytoplasm to a finely granular, homogeneous type, and cytoplasmic vacuolation. At $600 mg/kg/day many of the livers had vacuolation of centrolobular hepatocytes and some had hepatocyte necrosis. Examinations performed at 8 weeks and 6 months postexposure showed that the liver effects persisted in the rats exposed to $50 mg/kg/day for 13 weeks (IRDC 1977).

PentaBDE induced liver effects in rats exposed to $9 mg/kg/day for 28 days (increased liver weight and enlargement of centrolobular and midzonal liver parenchymal cells) (IRDC 1976) and $2 mg/kg/day for 90 days (hepatocytomegaly) (WIL Research Laboratories 1984). The hepatomegaly in the 90-day study was dose-related with respect to severity (some affected hepatocytes at higher doses had vacuoles that likely contained lipid) and not completely reversible, as it was still evident in $10 mg/kg/day males and 100 mg/kg/day females at 24 weeks postexposure in lessened severity and incidence. Females exposed to 2 and 100 mg/kg/day pentaBDE for 90 days had an increased incidence of degeneration and necrosis of individual liver parenchymal cells at 24 weeks postexposure; the investigators concluded that this may represent the final loss of previously damaged cells and probably should be considered compound-related (WIL Research Laboratories 1984). The 2 mg/kg/day LOAEL for hepatic effects of pentaBDE was used as the basis for the intermediate-duration MRL for oral exposure as indicated in the footnote to Table 3-2 and discussed in Chapter 2 and Appendix A. Liver vitamin A concentrations were increased in rats and

mice exposed to pentaBDE by gavage in doses of 18 and 36 mg/kg/day, respectively, for 90 days (Hallgren et al. 2001).

Hepatic microsomal enzyme induction is a well-documented effect of PBDEs. Microsomal enzyme activity was induced in rats exposed by gavage to doses as low as 0.6 mg/kg/day of octaBDE and 0.4 mg/kg/day of pentaBDE for 90 days as indicated by increases in O-ethyl O-p-nitrophenyl phenylphosphonothioate (EPN) detoxification, *p*-nitroanisole demethylation, and cytochrome c reductase and cytochrome P-450 levels (Carlson 1980a). Some of these changes were persistent, lasting for 30-60 days after cessation of treatment, but not considered to be adverse due to the lack of any accompanying hepatic histological abnormalities. Rats that were treated with equimolar (0.1 mmol/kg/day) gavage doses of deca-, octa-, or pentaBDE (95.9, 76.6, or 56.4 mg/kg/day, respectively) for 14 days had octa- and pentaBDE-induced increases in liver weight and microsomal enzyme activity (e.g., increased EPN detoxification, *p*-nitroanisole demethylation, uridinediphosphate-glucuronyltransferase [UDPGT] activity, and benzo[a]pyrene hydroxylase activity); exposure to decaBDE only increased liver weight (Carlson 1980b). DecaBDE also had no effect on hepatic UDPGT, ethoxyresorufin-o-deethylase (EROD), or pentoxyresorufin-o-deethylase (PROD) activities in weanling rats that were treated with #100 mg/kg/day by gavage for 4 days (Zhou et al. 2001). Similar exposure to 0.3–300 mg/kg/day pentaBDE caused significantly increased EROD and PROD at $10 mg/kg/day and UDPGT at $30 mg/kg/day, and octaBDE induced PROD at $10 mg/kg/day and EROD and UDPGT at $30 mg/kg/day; neither of these PBDEs caused induction at #1 mg/kg/day.

Hepatic EROD, PROD, and methoxyresorufin-o-deethylase (MROD) were increased in mice exposed to $18 mg/kg/day (lowest tested dose) of pentaBDE by gavage for 14 days, although UDPGT was unchanged at #36 mg/kg/day (Fowles et al. 1994; Hallgren et al. 2001). Rats that were similarly treated with pentaBDE for 14 days had increased activities of EROD, MROD, and PROD at $18 mg/kg/day (lowest tested dose) and increased UDPGT at 36 mg/kg/day (Hallgren et al. 2001). PentaBDE also increased hepatic microsomal enzyme activity in maternally-exposed rats and their offspring (Zhou et al. 2002). Exposure to 1, 10, or 30 mg/kg/day by gavage from gestation day (Gd) 6 through postnatal day (Pnd) 21 caused significantly increased hepatic EROD and PROD at $10 mg/kg/day in dams (Gd 20 and Pnd 22) and offspring (Gd 20 and Pnds 4, 14, and 36), as well as increased UDPGT at 30 mg/kg/day in dams (Gd 20 and Pnd 22) and offspring (Gd 20 and Pnds 4 and 14).

Renal Effects.

Polybrominated Biphenyls. No statistically significant correlations were found between serum PBB levels and serum levels of blood urea nitrogen (BUN) or creatinine in a study of residents of quarantined Michigan farms after the 1973 PBB contamination episode (Kreiss et al. 1982). No other studies were located with information pertinent to renal effects in humans after oral exposure to PBBs.

Studies with animals have shown some renal effects following prolonged exposure to PBBs, but findings are generally inconsistent, and the functional significance is uncertain. No exposure-related histological changes in kidneys or bladder were observed in rats administered FireMaster FF-1 in a single dose of 200 or 1,000 mg/kg (Kimbrough et al. 1978b, 1981) and observed for 2–23 months posttreatment (Kimbrough et al. 1978a, 1981), or #30 mg/kg/day for 2 weeks (Gupta et al. 1981). Gross examination of rats exposed to 1,000 mg/kg/day for 2 weeks showed darkened kidneys (Gupta and Moore 1979). Other renal information was not reported, but the dosage was lethal. Urinalysis was normal in rats following exposure to #30 mg/kg/day for 2 weeks (Gupta et al. 1981); urinalysis was not evaluated in the other rat studies. Bladder histology, examined in some of the rat studies, was also reported to be normal (Gupta et al. 1981; Kimbrough et al. 1981). Kidney histology was not altered in rats exposed to #71 mg/kg/day octabromobiphenyl mixture for 2 weeks (Lee et al. 1975b; Waritz et al. 1977). Mice exposed to #30 mg/kg/day FireMaster FF-1 for 2 weeks showed no abnormal kidney or bladder histology or urinalysis findings (Gupta et al. 1981). Information on acute-duration renal effects in other species was not located.

In intermediate-duration studies with rats, dietary exposure to FireMaster BP-6 for 30 days produced no PBB-related alterations in urinalysis indices or BUN at 5 mg/kg/day (highest tested dose) or kidney histology at #10 mg/kg/day (Akoso et al. 1982a; Sleight et al. 1978). However, 5 mg/kg/day FireMaster BP-6 in the diet for 3 months caused progressive obsolescence of glomeruli in rats (Bowman's membrane was shrunken and glomerular tufts were shrunken, inactive, or had been largely replaced by scar tissue), although relative kidney weight, BUN, and renal function tests (clearance of inulin, *p*-aminohippurate, or fractional sodium excretion) were normal (McCormack et al. 1978). Also, *in vitro* accumulation of p-aminohippurate and N-methylnicotinamide, and ammoniagenesis and gluconeogenesis were not affected in renal cortical slices from these treated rats. Administration of FireMaster FF-1 by gavage for 25 weeks caused no renal effects at 0.1 mg/kg/day, but produced chronic progressive nephropathy at $1 mg/kg/day, and more serious histopathology at 10 mg/kg/day (NTP 1983). Renal pathology at the 10 mg/kg/day dosage included atrophy and edema of glomerular tufts with marked dilation of Bowman's

capsule and dilation of some renal tubules, with either serous fluid or proteinaceous casts in both cortical and medullary regions, and no changes in BUN (NTP 1983). Chronic administration of FireMaster FF-1 in the diet for up to 104 weeks, however, failed to produce any treatment-related histopathologic changes at dosages as high as 1.5 mg/kg/day (NTP 1992). The reason for the inconsistency between this finding and the results of the NTP (1983) study is unclear, but the different methods of oral treatment could be a factor.

Intermediate-duration gavage exposure to a higher FireMaster FF-1 dose of 30 mg/kg/day for 4.5 weeks caused dilation of Bowman's capsule with serous fluid in rats observed for . 60 days posttreatment (Gupta and Moore 1979); however, rats that were similarly treated (#30 mg/kg/day for 30 days) but observed longer (90 days posttreatment) had normal kidney histology, urinalysis values, and BUN (Gupta et al. 1981). Rats administered 50 mg/kg/day of an unspecified PBB mixture in the diet for 30 days with no posttreatment observation had increased BUN but no changes in urinalysis values or kidney histology (Sleight and Sanger 1976). In studies with octabromobiphenyl mixture in rats, dietary exposure to $8 mg/kg/day for 30 days caused hyaline degenerative cytoplasmic changes in kidneys with normal urinalysis values (Norris et al. 1975a). This finding is inconsistent with a report of normal kidney histology in rats exposed to #71 mg/kg/day octabromobiphenyl mixture in the diet for 4 weeks (urinalysis not constructed) (Lee et al. 1975b; Waritz et al. 1977); the reason for the discrepancy cannot be discerned from the reports. Kidney histology and urinalysis findings were also normal in rats administered #1 mg/kg/day octabromobiphenyl mixture for 8 months (Norris et al. 1975a). In the only study of a decabromobiphenyl mixture, urinalysis was normal in rats fed 100 mg/kg/day for 13 weeks (Millischer et al. 1980). Comprehensive histology evaluations were performed at this and lower dosages in this study, but the liver is the only tissue specifically mentioned as having been examined. Due to the total number tissues examined (21) and the route of exposure, it is probable that kidneys were examined but were not discussed because no histopathologic changes were found.

In gavage studies with mice, FireMaster FF-1 produced no renal histopathologic changes following exposure to #10 mg/kg/day for 25 weeks (BUN was normal) (NTP 1983) or #30 mg/kg/day for 30 days (normal BUN and urinalysis) (Gupta et al. 1981). Dietary exposure to 3.9 mg/kg/day FireMaster FF-1 for up to 105 weeks, however, caused an increased incidence of chronic progressive nephropathy in mice; this effect was not found at 1.3 mg/kg/day (NTP 1992). Kidney histological alterations were not reported in mink exposed to #2.4 mg/kg/day FireMaster FF-1 for 313 days (Aulerich and Ringer 1979; Ringer et al. 1981). Swine exposed to unspecified PBBs for 16 weeks had increased relative kidney weight at $1 mg/kg/day, but the adversity of this change is unclear since no gross renal pathology was observed

(#8 mg/kg/day) and histology was not evaluated (Ku et al. 1978). Monkeys that ingested 18 mg/kg/day FireMaster FF-1 for 137 days developed hyperplasia of the bladder epithelium, but histological changes in the kidneys were not reported (Allen et al. 1978). Urine alterations in six pregnant cows given 67 mg/kg/day of FireMaster BP-6 in capsules for up to 60 days included increased protein concentration and BUN, and decreased pH and specific gravity (Moorhead et al. 1977). Necropsy following sacrifice between days 33 and 66 because of impending death due to poor health showed enlarged, distended, and discolored kidneys, extreme dilation of the collecting ducts and convoluted tubules, degenerative changes in the tubular epithelium, and congestion with scattered microscopic hemorrhages in the medulla. The renal effects in cows appear to be more severe than those generally observed in other species at lethal doses. No urinalysis alterations or changes in kidney histology were observed in other cows treated with #0.65 mg/kg/day and examined 1 or 140 days following the end of treatment.

Polybrominated Diphenyl Ethers. No studies were located regarding renal effects in humans after oral exposure to PBDEs.

Studies of PBDEs in animals have mainly observed kidney changes following dietary exposure to octaBDE. Renal effects induced by octaBDE included hyaline degenerative cytoplasmic changes at $8 mg/kg/day and increased kidney weight at $80 mg/kg/day in rats exposed in the diet for 30 days (Norris et al. 1973, 1975a, 1975b), and noninflammatory kidney changes in male rats exposed to 600 mg/kg/day for 13 weeks (IRDC 1977). The incidence and severity of the kidney lesions in the 13-week study (tubule regeneration, intratubular casts, and cellular debris occurred in most 600 mg/kg/day males) suggested a compound-related effect (IRDC 1977). Another dietary study of octaBDE found no histopathological changes in the kidneys of rats exposed to #90 mg/kg/day for 90 days (IRDC 1976). Kidney pathology (hyaline degenerative cytoplasmic changes) was found in rats exposed to 800 mg/kg/day of decaBDE for 30 days (Norris et al. 1973, 1975a, 1975b), but not following exposure of rats to #90 mg/kg/day for 90 days (IRDC 1976), #8,000 mg/kg/day for 13 weeks (NTP 1986), #1.0 mg/kg/day (77.4% containing 21.8% nonaBDE and 0.8% octaDBE) for 2 years (Kociba et al. 1975; Norris et al. 1975b), or #2,550 mg/kg/day for 103 weeks (NTP 1986), or in mice exposed to #9,500 mg/kg/day for 13 weeks or #7,780 mg/kg/day for 103 weeks (NTP 1986). Studies of pentaBDE found no renal histopathology in rats exposed dietary doses of #90 mg/kg/day for 28 days (IRDC 1976) or #100 mg/kg/day for 90 days (WIL Research Laboratories 1984).

Endocrine Effects.

Polybrominated Biphenyls. No studies were located regarding endocrine effects in humans after oral exposure to PBBs.

Thyroid effects have been observed in animals treated with PBBs by gavage or diet in acute-, intermediate-, and chronic-duration studies. Characteristic changes include decreases in serum levels of serum thyroxine (T_4) and serum triiodothyronine (T_3) hormones, thyroid enlargement, and effects in the follicular cells including reduced size, hyperplasia with columnar appearance and papillary projections, and accumulation of colloid droplets. In the only acute study that investigated thyroid end points more sensitive than histology, rats administered an unspecified PBB mixture for 10 days showed serum T_4 levels (T_3 not evaluated) that were significantly reduced (p#0.05) at $3 mg/kg/day, but not at 1 mg/kg/day (Allen-Rowlands et al. 1981). The reduction in T_4 levels was both dose- and time-dependent as shown by 20-day results discussed below with intermediate-duration studies. Based on the NOAEL for decreased serum T_4, an acute oral MRL of 0.01 mg/kg/day was calculated as described in the footnote in Table 3-1. A single #286 mg/kg dose of an unspecified PBB mixture caused no change in 4-hour thyroidal ^{131}I uptake and incorporation into thyroglobulin in rats (Allen-Rowlands et al. 1981). No thyroid histological alterations were observed in rats in acute-duration studies with FireMaster FF-1, even with a single dose #1,000 mg/kg and up to 2 years posttreatment observation (Kimbrough et al. 1978b, 1981) or dosages of #1,000 mg/kg/day for 2 weeks (Gupta and Moore 1979; Gupta et al. 1981). The only information on thyroid effects of acute exposure to octabromobiphenyl mixture is a lack of histological changes in rats administered #71 mg/kg/day for 2 weeks (Lee et al. 1975b; Waritz et al. 1977). The only information on acute-duration thyroid effects of PBBs in species other than rat is the normal histologic integrity of the thyroid in mice at FireMaster FF-1 dosages of #30 mg/kg/day for 2 weeks (Gupta et al. 1981).

In intermediate-duration studies with rats, serum levels of T_3 or T_4 decreased at FireMaster dosages as low as 0.3 mg/kg/day FireMaster FF-1 for 25 weeks (NTP 1983), 0.45 mg/kg/day FireMaster BP-6 for 7 months (Byrne et al. 1987), 5 mg/kg/day FireMaster BP-6 for 30 days (Akoso et al. 1982b) or 1 mg/kg/day of an unspecified PBB mixture for 20 days (Allen-Rowlands et al. 1981). In the latter study, 8–11 rats were evaluated after exposure to 1, 3, or 6 mg/kg/day for 20 days. Other thyroid effects in these rats included significantly increased absolute thyroid weight at $3 mg/kg/day (not evaluated at 1 mg/kg/day), and increased plasma TSH levels, increased 5-hour thyroid uptake of ^{131}I and deceased incorporation of ^{131}I into monoiodotyrosine (MIT) at 6 mg/kg/day (Allen-Rowlands et al. 1981). No

effects on incorporation of ^{131}I into diiodotyrosine (DIT), T_3, or T_4 were observed. Serum T_4 levels were also reduced at $1 mg/kg/day in rats exposed for 20 days and evaluated after being placed on restricted food intake for $2 months following treatment (Allen-Rowlands et al. 1981). Rats administered 2.5 mg/kg/day of an unspecified hexabromobiphenyl mixture for 7 months showed no significant changes in serum T_3, but serum T_4 was not evaluated (Sepkovic and Byrne 1984). Thyroid ultrastructural changes were produced in rats by FireMaster BP-6 dosages as low as 0.05 mg/kg/day for 30–35 days (Akoso et al. 1982b; Kasza et al. 1978a), and histologic changes of the thyroid were observed at $5 mg/kg/day FireMaster BP-6 for 30 days (Sleight et al. 1978) and $30 mg/kg/day FireMaster FF-1 for 4.5 weeks (Gupta and Moore 1979). In the study that evaluated thyroid effects at the lowest dose, rats were administered estimated doses of 0.05, 0.5, or 5 mg/kg/day FireMaster BP-6 in the diet for 30 days (Akoso et al. 1982b). Effects were dose-dependent and included increased number and decreased size of follicles (especially at the peripheral location) at $0.05 mg/kg/day, follicles with epithelial tall columnar appearance and some papillary projections in the lumen at $0.5 mg/kg/day, and extensive follicular changes (hyperplasia and hypertrophy of follicular cells, prominent, and numerous papillary projections), increased relative thyroid weight, and decreased serum T_3 and T_4 at 5 mg/kg/day. Chronic exposure to #1.5 mg/kg/day FireMaster FF-1 for up to 104 weeks caused no thyroid histological alterations, but ultrastructure and serum thyroid hormones were not assayed (NTP 1992).

In the only intermediate-duration rat study of octabromobiphenyl mixture that assessed thyroid hormones, a dose of 2.5 mg/kg/day for 7 months produced no significant changes in serum T_3, but serum T_4 was not evaluated (Sepkovic and Byrne 1984). The histologic integrity of the thyroid was normal in rats fed octabromobiphenyl mixture at dosages as high as 1 mg/kg/day for 8 months (Norris et al. 1975a), 2.5 mg/kg/day for 7 months (Sepkovic and Byrne 1984), and 71 mg/kg/day for 4 weeks (Lee et al. 1975b; Waritz et al. 1977), although $8 mg/kg/day for 30 days induced dose-related thyroid hyperplasia (Norris et al. 1975a). An explanation for the discrepancy in the octabromobiphenyl mixture NOAELs of #71 mg/kg/day and LOAELs of $8 mg/kg/day is not apparent, particularly since treatment durations were similar, methods of treatment (diet) and animal strain and sex (male) were the same, and only the NOAEL study appears to have observed the animals (for 2–18 weeks) posttreatment.

Effects on the adrenal gland also have been observed in animals exposed to PBBs. As found for thyroid as discussed above, acute-duration exposure to FireMaster FF-1 produced no changes in rat adrenal histology following a single dose as high as 1,000 mg/kg (Gupta et al. 1981; Kimbrough et al. 1978b, 1981). Dosages of 1,000 mg/kg/day FireMaster FF-1 for 2 weeks caused gross adrenal damage (darkened glands) in rats, but #30 mg/kg/day caused no gross or histologic damage (Gupta and Moore 1979; Gupta

et al. 1981). The only information on acute-duration adrenal effects of PBBs in species other than rat is normal adrenal histology in mice at FireMaster FF-1 dosages of #30 mg/kg/day for 2 weeks (Gupta et al. 1981). No acute-duration studies of PBBs measure serum corticosteroid levels. In intermediate-duration studies, serum corticosterone B, dehydroepiandrosterone (DHEA), and dehydroepiandrosterone sulfate (DHS) decreased in rats fed $0.25 mg/kg/day FireMaster BP-6 for 5–7 months, but not 0.05 mg/kg/day (Byrne et al. 1988). Serum corticosterone levels and adrenal weight did not change in rats exposed to #6 mg/kg/day of an unspecified PBB mixture for a shorter duration of 20 days (other adrenal hormones were not evaluated) (Castracane et al. 1982). Adrenal histology was not evaluated in these studies, but no treatment-related alterations were observed in rats in other intermediate-duration studies with FireMaster BP-6 or FireMaster FF-1 (Akoso et al. 1982b; NTP 1983; Sleight and Sanger 1976; Sleight et al. 1978), except at lethal dosages (Gupta and Moore 1979), or in a chronic study with FireMaster FF-1 (NTP 1992). Necropsies of rats treated with 100–1,000 mg/kg/day FireMaster FF-1 for 4.5 weeks showed darkened adrenals (Gupta and Moore 1979). In the only rat study of octabromobiphenyl mixture that examined the adrenal gland, 2.5 mg/kg/day for 7 months produced no changes in relative adrenal weight; histology or serum corticosteroids were not evaluated (Sepkovic and Byrne 1984). Intermediate- or chronic-duration studies with FireMaster FF-1 in mice showed no adrenal histological effects at #3.9 mg/kg/day for up to 105 weeks (NTP 1992), #10 mg/kg/day for 25 weeks (NTP 1983), or #30 mg/kg/day for 30 days (Gupta et al. 1981).

Polybrominated Diphenyl Ethers. Plasma levels of 2,2',4,4'-tetraBDE and various other persistent organohalogen compounds (non-PBDEs), as well as hormone levels (free and total T_3 and T_4, thyroid stimulating hormone [TSH], free testosterone, follicle-stimulating hormone, lutenizing hormone, and prolactin), were analyzed in 110 men who consumed varying amounts of fatty fish (0–32 meals per month) from the Baltic Sea (Hagmar et al. 2001). There was a weak negative correlation between 2,2',4,4'-tetraBDE and plasma TSH after age adjustment, but 2,2',4,4'-tetraBDE could not explain more than 10% of the variance in TSH (r^2=0.10, $p<0.001$).

Hyperplasia of the thyroid has been observed in rats and mice following repeated dietary exposures to decaBDE. Thyroid follicular cell hyperplasia was increased in male B6C3F1 mice that were exposed to decaBDE for 103 weeks (NTP 1986). Incidences of the lesion were 2/50 (4%), 10/50 (20%), and 19/50 (38%) in the 0, 3,200, and 6,650 mg/kg/day dose groups of this study. Slight increases in follicular cell tumors that were considered to be equivocal evidence of thyroid carcinogenicity were also observed in the male mice (see Section 3.2.2.7, Cancer). No decaBDE-related histopathological changes in the thyroid were found after 103 weeks of exposure to #7,780 mg/kg/day in female mice, #2,240 mg/kg/day

in male Sprague-Dawley rats, or #2,550 mg/kg/day in female rats (NTP 1986). Dose-related increases in thyroid hyperplasia were reported for male Sprague-Dawley rats exposed to 80 and 800 mg/kg/day for 30 days (Norris et al. 1973, 1975b), although not in rats exposed to #90 mg/kg/day for 90 days, rats exposed to #8,000 mg/kg/day for 13 weeks, or mice exposed to #9,500 mg/kg/day for 13 weeks (IRDC 1976; NTP 1986). The occurrence of thyroid hyperplasia in the rats exposed to $80 mg/kg/day for 30 days could be related to the congener composition of the decaBDE mixture tested by Norris et al. (1973, 1975a, 1975b) (i.e., 77.4% decaBDE, 21.8% nonaBDE, and 0.8% octaBDE, compared to $94% decaBDE typically used in other studies).

Thyroid hormone levels were determined in weanling (28-day-old) female Long-Evans that were treated by gavage for 4 days with technical decaBDE (DE-83R) or octaBDE (DE-79) in doses of 0.3, 1, 3, 10, 30, 60, or 100 mg/kg/day, or pentaBDE (DE-71) in doses of 0.3, 1, 3, 10, 30, 100, or 300 mg/kg/day (Zhou et al. 2001). The animals were sacrificed on the day after the last exposure and evaluated for changes in serum levels of total T_4, total T_3, and thyroid-stimulating hormone (TSH). DecaBDE caused no changes in levels of any of the thyroid hormones. OctaBDE induced a dose-related reduction in serum T_4 levels with statistically significant ($p<0.05$) decreases occurring at $10 mg/kg/day and a 70% maximum decrease compared to controls at the highest dose of 100 mg/kg/day. Serum total T_3 levels were significantly reduced at $60 mg/kg/day with a maximum reduction of 25% at 100 mg/kg/day. PentaBDE caused dose-related reductions in serum T_4 levels with significant decreases occurring at $30 mg/kg/day and an 80% maximum decrease compared to controls at the highest dose of 300 mg/kg/day. Serum total T_3 levels were significantly reduced at $100 mg/kg/day with a maximum reduction of 30% at 300 mg/kg/day. Neither octaBDE nor pentaBDE caused exposure-related changes in serum TSH concentrations. Benchmark dose (BMD) analysis of the octaBDE data found that the BMD and BMDL (95% lower confidence limit on the BMD) resulting in a 20% reduction in thyroid hormones (LED_{20}) were 9.25 and 5.29 mg/kg/day, respectively, for serum T_4 and 53.38 and 11.98 mg/kg/day, respectively, for serum T_3. For pentaBDE, the respective BMD and BMDL resulting in 20% reduced hormone levels were 12.74 and 6.95 mg/kg/day for serum T_4 and 32.94 and 8.56 mg/kg/day for serum T_3.

Serum levels of thyroid hormones were not evaluated in any other study of octaBDE. Dose-related increases in thyroid hyperplasia were observed in male Sprague-Dawley rats (incidences not reported) exposed to $8 mg/kg/day (lowest tested dose) in the diet for 30 days (Norris et al. 1973, 1975b). Thyroid hyperplasia was equivocally increased in male CD rats that were exposed to 90 mg/kg/day of octaBDE (unspecified mixture) in the diet for 28 days (IRDC 1976); incidences of slight or moderate hyperplasia were 0/5, 0/5, and 3/5 at 0, 9, and 90 mg/kg/day, respectively. CD rats that were exposed to octaBDE in

estimated dietary doses of 5, 50, or 600 mg/kg/day (males) or 7, 70, or 750 mg/kg/day (females) for 13 weeks had increased absolute and relative thyroid weights at $50/70 mg/kg/day (IRDC 1977). The thyroid weight increases were still observed at 8 weeks postexposure in the 600/750 mg/kg/day groups and were concluded to be likely compound-related. Most of the follicles in the thyroids of 4/35 males at 600 mg/kg/day and 1/35 females at 750 mg/kg/day had epithelium that was tall columnar rather than the normal cuboidal type. This effect was considered to be a very slight but probably compound-related histological change. The thyroid glands were considered within the range of normal morphology at 8 weeks postexposure in the 600/750 mg/kg/day rats as well as in the lower dose groups (IRDC 1977).

The 4-day study in weanling rats summarized above (Zhou et al. 2001) is not the only study of pentaBDE that found suppressed thyroid hormone levels. Serum T_4 levels were also reduced by pentaBDE in maternally-exposed rats and their offspring (Zhou et al. 2002). Long-Evans rats were administered 1, 10, or 30 mg/kg/day of pentaBDE (DE-71) in corn oil by gavage from Gd 6 through Pnd 21. Total concentrations of serum T_4 and T_3 were evaluated in dams on Gd 20 and Pnd 22 and offspring were evaluated on Gd 20 and Pnds 4, 14, 36, and 90. Serum T_4 was significantly ($p<0.05$) reduced compared to controls in dams at 30 mg/kg/day on Gd 20 and Pnd 22 (48 and 44% reduced, respectively), and in offspring at $10 mg/kg/day on Gd 20 ($15% reduced) and Pnds 4 and 14 (50 and 64% reduced at 10 and 30 mg/kg/day, respectively). The effect on serum T_4 concentrations in the offspring returned to control levels by Pnd 36. The NOAEL for the effect on serum T_4 levels (1 mg/kg/day) was used as the basis for the acute-duration MRL for oral exposure as indicated in the footnote to Table 3-2 and discussed in Chapter 2 and Appendix A. There were no exposure-related changes in serum T_3 levels in the dams or offspring, or any significant effects of treatment on litter size, sex ratio, or nonneurodevelopmental measures of offspring viability and growth as discussed in Section 3.2.2.6 (Developmental Effects). The BMD and BMDL resulting in a 20% reduction in serum T_4 levels were determined to be 2.36 and 0.94 mg/kg/day, respectively.

Serum total T_4 concentrations were significantly reduced in female C57BL/6J mice that were given a single gavage dose of pentaBDE (DE-71) as low as 0.8 mg/kg (lowest tested dose) (Fowles et al. 1994). Reductions in serum T_4 were found in four of five dose groups (i.e., 0.8, 4, 20, and 500 mg/kg, but not 100 mg/kg), but did not occur in a dose-dependent manner. This lack of dose-dependency might be explained by the number of animals per group, which was relatively small (n=6). Evaluation of mice exposed to 18, 36, or 72 mg/kg/day of pentaBDE by daily gavage for 14 days showed significantly reduced total and free T_4 levels at $18 mg/kg/day (Fowles et al. 1994). Serum T_3 and TSH levels and thyroid histology were not evaluated in the single-dose and 14-day mouse studies. Serum levels of total

and free T_4 were also significantly reduced in female C57BL/6J mice as well as female Sprague-Dawley rats that were similarly exposed to $18 mg/kg/day (lowest tested dose) of a different pentaBDE mixture (Bromkal 70-5 DE) for 14 days (Darnerud and Sinjari 1996; Hallgren et al. 2001). There were no exposure-related changes in TSH levels in the rats or mice and serum T_3 was not evaluated in either species. Serum measurements in rats and mice that were treated with 1, 6, or 18 mg/kg/day of 2,2',4,4'-tetraBDE for 14 days showed significantly reduced T_4 levels in both species at 18 mg/kg/day (Darnerud and Sinjari 1996; Hallgren and Darnerud 1998). Exposure to 18 mg/kg/day of 2,2',4,4'-tetraBDE did not affect TSH levels in the rats (not determined in mice).

End points assessed in a comprehensive 90-day feeding study of pentaBDE (DE-71) in male and female Sprague-Dawley rats included serum T_3 and T_4 levels (TSH not measured) and thyroid histology (WIL Research Laboratories 1984). Effects observed in both sexes included significantly reduced plasma T_4 levels at $10 mg/kg/day and increased follicular cell hyperplasia at 100 mg/kg/day. Incidences of follicular cell hyperplasia in the 0, 2, 10, and 100 mg/kg/day dose groups of this study were 0/10, 2/10, 2/10, and 5/10 in males and 0/10, 0/10, 1/10, and 4/10 in females. The thyroid hyperplasia was mild and transient as it was characterized as very slight in severity at all doses and was no longer observed at 24 weeks postexposure in any animals. Thyroid hyperplasia was equivocally increased in male CD rats that were exposed to 90 mg/kg/day of pentaBDE (unspecified mixture) in the diet for 28 days (IRDC 1976). Incidences of slight or moderate hyperplasia in the 0, 9, or 90 mg/kg/day dose groups of this study were 0/5, 1/5, and 3/5 males, respectively; no increases were seen in females.

Information on hormonal effects of PBDEs other than thyroid is limited to adrenal corticosterone data from a 14-day study of pentaBDE (DE-71). Measurements of serum corticosterone levels in female mice showed no clear chemical-related changes following exposure to 18, 36, or 72 mg/kg/day by daily gavage for 14 days (Fowles et al. 1994).

Dermal Effects.

Polybrominated Biphenyls. Limited human data from an epidemiological study provide suggestive evidence that oral exposure to PBBs may produce skin disorders in humans, but do not provide information regarding dose-response relationships. Symptoms of skin disorders (rashes, acne, increased sensitivity to the sun, darkening or thickening of the skin, discoloration or deformity of fingernails or toenails, slow healing of cuts) were reported with greater frequency in a group of 406 Michigan residents probably exposed to PBBs than in a group of 153 likely unexposed residents, but no association was

evident between serum PBB levels and prevalence of skin disorders (Anderson et al. 1978c). In a medical history survey study conducted in 1976, symptoms of skin disorders (peeling and scaling, erythema, hair loss, increased nail growth, increased sweating) experienced during the previous 3 years were reported at higher prevalence rates in a group of 321 Michigan residents from quarantined farms and in a group of 177 nonquarantined farm residents than in a group of 149 nonexposed Wisconsin residents (Chanda et al. 1982). Physical examination of the combined group of Michigan residents revealed alopecia in 4% of the subjects compared to no occurrence of alopecia in the control group.

PBBs produced characteristic dermal changes in certain species of animals following repeated exposures. No exposure-related histological changes were observed in the skin, salivary glands, or eyes of rats administered a single dose of 200 mg/kg FireMaster FF-1 and observed for 18–22 months (Kimbrough et al. 1981). Rats and mice exposed to #30 mg/kg/day FireMaster FF-1 for 2 weeks showed no histological alterations in pinnae, ear canals, or salivary glands, but examination of skin was not performed (Gupta et al. 1981). In intermediate- and chronic-duration studies, rats and/or mice exposed to FireMaster FF-1 or FireMaster BP-6 dosages of #30 mg/kg/day by gavage for 30 days (Gupta et al. 1981), #10 mg/kg/day in the diet for 30 days (Akoso et al. 1982a), #10 mg/kg/day by gavage for 25 weeks (NTP 1983), or #3.9 mg/kg/day in the diet for up to 105 weeks (Chhabra et al. 1993; NTP 1992) showed no histopathological changes in skin, pinnae, ear canals, or salivary glands. Xerophthalmia (extreme dryness of the conjunctiva, with keratinization of epithelium following chronic conjunctivitis) was observed in rats after 82 days of dietary exposure to 5 mg/kg/day FireMaster BP-6 (Darjono et al. 1983). Alopecia, loss of eyelashes, generalized subcutaneous edema, dry scaly skin, and periorbital edema developed in monkeys exposed to FireMaster FF-1 in dosages of $0.73 mg/kg/day for $25 weeks (two animals) or 18 mg/kg/day for 137 days (one animal) (Allen et al. 1978; Lambrecht et al. 1978). Histological examination, performed only in the monkey exposed to 18 mg/kg/day, showed atrophy and squamous metaplasia of sebaceous glands and keratinization of hair follicles (Allen et al. 1978). Dermatosis on the ventral surface was a clinical sign in two of four swine administered 8 mg/kg/day FireMaster FF-1 for 16 weeks (Ku et al. 1978). No additional information was reported on the dermatosis (a nonspecific term used to denote any cutaneous lesion or group of lesions), and histologic examinations were not completed.

Polybrominated Diphenyl Ethers. No studies were located regarding dermal effects in humans after oral exposure to PBDEs.

Histopathological examinations showed no dermal changes in rats following dietary exposure to #750 mg/kg/day of octaBDE for 13 weeks (IRDC 1977) or #100 mg/kg/day of pentaBDE for 90 days (WIL Research Laboratories 1984).

Ocular Effects.

Polybrominated Biphenyls. No studies were located regarding ocular effects in humans after oral exposure to PBBs.

Occasional eye discharge was observed in pregnant cows that were administered 67 mg/kg/day of FireMaster BP-6 in capsules for up to 60 days (Moorhead et al. 1977). Necropsy following sacrifice between days 33 and 66 because of impending death due to poor health showed hyperkeratosis of the eyelids and squamous metaplasia with keratin cysts in the tarsal glands in five of six animals. No ocular effects were observed in other cows treated with #0.65 mg/kg/day and examined 1 or 140 days following the end of treatment. Histological changes were not observed in the eyes of rats exposed to FireMaster FF-1 for 2 weeks (Gupta et al. 1981), or in rats and mice treated by gavage (NTP 1983) or fed FireMaster FF-1 for up to 105 weeks (Chhabra et al. 1993; NTP 1992).

Polybrominated Diphenyl Ethers. No studies were located regarding ocular effects in humans after oral exposure to PBDEs.

Histopathological examinations showed no ocular effects in rats following dietary exposure to #1.0 mg/kg/day of 77.4% decaBDE (containing 21.8% nonaBDE and 0.8% octaDBE) for 2 years (Kociba et al. 1975; Norris et al. 1975b), #750 mg/kg/day of octaBDE for 13 weeks (IRDC 1977), or #100 mg/kg/day of pentaBDE for 90 days (WIL Research Laboratories 1984).

Body Weight Effects.

Polybrominated Biphenyls. No studies were located regarding body weight effects in humans after oral exposure to PBBs.

Reduced body weight was observed in various species following acute oral administration of relatively high doses of PBBs; this effect is most evident with repeated exposure. In general, decreases in food and water intake are not sufficient to account for decreases in body weight. Effects on body weight can be

quite pronounced following intermediate- and chronic-duration exposure, constituting a wasting syndrome manifested by weight loss and depletion of body fat. In acute-duration studies with rats, a single 1,000 mg/kg dose of FireMaster FF-1 caused decreased weight gain during the following 2 years (Kimbrough et al. 1981), and a single 800 mg/kg dose of FireMaster BP-6 during pregnancy caused maternal weight loss (Beaudoin 1977). Single FireMaster doses of 400 (BP-6) or 500 (FF-1) mg/kg/day did not affect body weight in rats (Beaudoin 1977; Kimbrough et al. 1980). Administration of 1,000 mg/kg/day FireMaster FF-1 or 130 mg/kg/day FireMaster BP-6 for 2 weeks produced decreased weight gain or weight loss in rats and mice, respectively (Corbett et al. 1978; Fraker 1980; Fraker and Aust 1980; Gupta and Moore 1979), but 5 mg/kg/day FireMaster BP-6 for 10 days had no effect on body weight in rats (Raber and Carter 1986). A single #2,000 mg/kg dose or two daily 3,000 mg/kg doses of octabromobiphenyl mixture had no effect on body weight gain in rats observed for the following 14–28 days (Lee et al. 1975a; Norris et al. 1975a). No changes in body weight were produced in rats exposed to #71 mg/kg/day octabromobiphenyl mixture for 2 weeks (Lee et al. 1975b; Waritz et al. 1977). In intermediate-duration studies, decreased body weight gain and/or weight loss has been observed in rats at dosages as low as 3 mg/kg/day FireMaster FF-1 for 6 months (Luster et al. 1980; NTP 1983), 5 mg/kg/day FireMaster BP-6 for 1–3 months (McCormack et al. 1978; Sleight et al. 1978), and 30–50 mg/kg/day FireMaster FF-1 for 4.5–5 weeks (Gupta and Moore 1979; Sleight and Sanger 1976). FireMaster FF-1 dosages $100 mg/kg/day for 4.5 weeks (lethal doses) caused weight loss and emaciation in rats (Gupta and Moore 1979). Final body weights were decreased $11–28% in rats exposed to 0.5 or 1.5 mg/kg/day FireMaster FF-1 for up to 104 weeks (NTP 1992). No body weight changes were observed in rats fed a decabromobiphenyl mixture at dosages as high as 100 mg/kg/day for 13 weeks (Millischer et al. 1980).

In mice exposed to FireMaster FF-1, estimated dosages of 10 mg/kg/day for 25 weeks (NTP 1992) and 21.7 mg/kg/day for 6 weeks (Loose et al. 1981) decreased the rate of weight gain. Chronic exposure to #3.9 mg/kg/day FireMaster FF-1 for up to 105 weeks, however, did not produce adverse effects on mouse body weight (NTP 1992). Guinea pig, mink, and monkey seem to be particularly sensitive species, as indicated by pronounced weight loss in guinea pigs from ingestion of 4 mg/kg/day of unspecified PBBs for 30 days (Sleight and Sanger 1976), decreased weight gain in mink at FireMaster FF-1 dosages as low as 0.39 mg/kg/day with weight loss at 1.86 mg/kg/day (Aulerich and Ringer 1979; Ringer et al. 1981), and weight loss in monkeys at FireMaster FF-1 dosages as low as 0.73 mg/kg/day for 25–50 weeks (Allen et al. 1978; Lambrecht et al. 1978). Monkeys that ingested an estimated FireMaster FF-1 dosage of 0.012 mg/kg/day for 66 weeks lost weight (Lambrecht et al. 1978). Food intake and body weight gain were reduced in pregnant cows after 4 and 20 days administration of 67 mg/kg/day FireMaster BP-6 in

capsules (Moorhead et al. 1977). This dosage was lethal because death was impending between days 33 and 66 (treatment duration was 60 days). There were no effects on food intake or body weight in cows treated with #0.65 mg/kg/day and observed 1 or 140 days following the end of treatment.

Polybrominated Diphenyl Ethers. No studies were located regarding body weight effects in humans after oral exposure to PBDEs.

DecaBDE had no effect on body weight gain in rats and mice that were exposed to dietary doses of #16,000 and #19,000 mg/kg/day, respectively for 14 days, #8,000 and #9,500 mg/kg/day, respectively, for 13 weeks (NTP 1986), or #2,550 and #7,780 mg/kg/day, respectively, for 103 weeks (NTP 1986). Dietary ingestion of 77.4% decaBDE mixture (containing 21.8% nonaBDE and 0.8% octaDBE) similarly caused no body weight changes in rats exposed to #800 mg/kg/day for 30 days or #1.0 mg/kg/day for 2 years (Kociba et al. 1975; Norris et al. 1973, 1975a, 1975b).

OctaBDE did not affect body weight in rats exposed to dietary doses of #90 mg/kg/day for 28 days (IRDC 1976) or #800 mg/kg/day for 30 days (Norris et al. 1973, 1975a, 1975b), although exposure to $600 mg/kg/day for 13 weeks caused $12% decreases in weight gain (IRDC 1977). There were no pentaBDE-related body weight changes in rats exposed to #90 mg/kg/day for 28 days (IRDC 1976) or mice exposed to #72 mg/kg/day pentaBDE for 14 days (Fowles et al. 1994), although the rate of weight gain was reduced in rats exposed to #100 mg/kg/day pentaBDE for 90 days (WIL Research Laboratories 1984).

3.2.2.3 Immunological and Lymphoreticular Effects

Polybrominated Biphenyls. Numerous reports have been published regarding the immunological competence of individuals exposed to PBBs in the Michigan feed contamination episode. Due to the relatively high number of published reports and to the fact that often different groups of investigators appear to have examined the same cohort, only representative studies are discussed below.

Immunological parameters were compared between a group of 45 adult Michigan dairy farmers and their families who were exposed for periods ranging from 3 months to 4 years and two groups of control individuals not known to have been exposed to PBBs (Bekesi et al. 1978, 1979). In 27 of the 45 Michigan subjects, the peripheral blood lymphocytes responded within a normal range to phytohemagglutinin (PHA) and PWM mitogen-induced lymphoblastogenesis, but had reduced reactivity in mixed leukocyte cultures relative to controls. In the remaining 18 Michigan subjects, the response to PHA and PWM and the reactivity to mixed leukocyte cultures was significantly reduced ($p<0.00001$) relative to controls. Assays for membrane markers of peripheral blood lymphocytes showed significant reductions in markers in the Michigan subpopulation with abnormal lymphoblastogenesis. Both Michigan subpopulations had a significant increase in the number of lymphocytes without detectable surface markers, relative to controls. The number of markers for monocytes was not significantly different among the groups studied. There were no significant differences in serum PBB levels between the two Michigan subsets. No consistent correlation could be demonstrated between lymphocyte function and PBB plasma concentration.

Reexamination of a group of 40 Michigan farmers 5 years after the first examination (Bekesi et al. 1985; Roboz et al. 1985) showed that the number of T-lymphocytes and the lymphocyte response to stimulation with PHA were altered to the same extent reported 5 years earlier (Bekesi et al. 1978).

In a similar study, Michigan subjects were classified into three groups according to their serum PBB levels: high (>300 ppb), low (<1–11 ppb), and unexposed (controls) (Silva et al. 1979). The percentage of subjects that complained of recurrent infection was similar in the two exposed groups (about 20%). The total leukocyte count, percentage of lymphocytes, and percentage of subpopulations of T- and B-lymphocytes were similar among the three groups. Mean spontaneous lymphocyte transformation and lymphocyte mitogenic responsiveness to stimulation with three different mitogens were not significantly different among the three groups. Furthermore, there was no correlation between a poor mitogenic response and low numbers of T-lymphocytes (Silver et al. 1979).

It was also reported at this time that Michigan farm residents with the highest exposure to PBB had significantly elevated levels of IgM, IgA, and IgG relative to Wisconsin dairy farm residents (Bekesi et al. 1985). Cluster analysis of several immunological parameters performed for husbands and wives showed, according to the investigators, significant correlations for surface markers, lymphocyte functions, and IgG values (no correlation coefficient was >0.337). This finding was interpreted as supporting a common dietary source for the immune dysfunction rather than a genetic predisposition (Bekesi et al. 1985).

In yet another report, Michigan farmers reported a higher rate of infections (11%) than a group of chemical workers exposed to PBB (3%) (Stross et al. 1981), however, average PBB levels in serum, bile, and fat were higher in the chemical workers than in the farmers. When the patients were divided according to their PBB fat level into high, moderate, and low, there was an equal distribution of abnormal physical, laboratory, and diagnostic findings among the groups.

The immunological effects of the commercial PBB mixtures FireMaster FF-1 and FireMaster BP-6 have been examined in rats, mice, guinea pigs, dogs, and pigs, but in many cases, the most sensitive immunological end points were not examined. In all but two studies, the animals were exposed for an intermediate duration; most studies were conducted in rats, and many studies administered the PBBs by gavage (exceptions noted below). Identification of the most sensitive species is difficult due to the fact that not all studies examined the same end points, although limited data suggest that guinea pigs may be particularly sensitive. Immunological effects in animals, attributed to exposure to PBBs *in utero* or through lactation, are discussed in Section 3.2.2.6.

Limited data exist regarding immunological effects of PBBs in animals following acute oral exposure. No histopathological alterations were observed in the spleen and thymus of rats treated with a single dose of 1,000 mg/kg FireMaster FF-1 and observed for 2 years (Kimbrough et al. 1978b). A similar lack of effects in the thymus, spleen, and lymph nodes was reported in rats and mice treated for 2 weeks with up to 30 mg/kg/day FireMaster FF-1 (Gupta et al. 1981). However, mice treated with . 130 mg/kg FireMaster BP-6 in the diet for 14 days were incapable of mounting an antibody-mediated response following immunization with sheep red blood cells (SRBC) (Fraker 1980; Fraker and Aust 1980). This treatment also reduced absolute thymus weight by 88% and caused high lethality in mice.

Numerous intermediate-duration studies have examined the immunological effects of PBBs in rats. For example, treatment of rats with FireMaster FF-1 for 25 weeks significantly increased absolute and relative spleen weight at $1 mg/kg/day and significantly decreased absolute and relative thymus weight at

$0.3 mg/kg/day (NTP 1983). Nevertheless, no histopathological alterations were observed in these organs and in lymph nodes with doses of up to 10 mg/kg/day (NTP 1983). Similar results were reported in rats treated with 30 mg/kg/day FireMaster FF-1 for 4–5 weeks (Gupta and Moore 1979; Gupta et al. 1981), but a dose of 100 mg/kg/day caused thymic atrophy and necrosis of lymphoblasts (Gupta and Moore 1979). A much smaller dose, 0.5 mg/kg/day FireMaster BP-6 in the diet for 150 days, reportedly caused moderate lymphoid depletion in thymus and spleen (Rezabek et al. 1989). In the only chronic rat study, splenic fibrosis developed following exposure to 1.5 mg/kg/day, but not 0.5 mg/kg/day, FireMaster FF-1 in the diet for up to 104 weeks (NTP 1992). Treatment of rats for 5 weeks with 30 mg/kg/day FireMaster FF-1 significantly reduced the *in vitro* lymphocytic response to stimulation with two out of three mitogens and thymus and spleen weight (Luster et al. 1978). Relative thymus weight was reduced at 3 mg/kg/day; however, treatment with the test material did not alter the production of antibodies 4 days after immunization with SRBC. The same group of investigators reported significantly decreased lymphoproliferative responses to mitogens or allogenic cells in rats following treatment with 3 mg/kg/day FireMaster FF-1 for 6 months (Luster et al. 1980); a dose of 1 mg/kg/day was without effect. It must be mentioned, however, that in the studies conducted by Luster and co-workers, doses $3 mg/kg/day FireMaster, reduced body weight by $15% in the animals, suggesting that PBBs can affect the immune system, but only at dose levels that produce overt toxicity.

Mice treated for 30 days with FireMaster BP-6 in the diet at levels of approximately $1.3 mg/kg/day had a significantly reduced antibody-mediated response to SRBC ($p<0.001$) (Fraker 1980; Fraker and Aust 1980). Absolute thymus weight was significantly reduced ($p<0.01$) relative to controls with all dose levels tested (0.13, 1.3, 13 mg/kg/day). Delayed-type hypersensitivity was not altered by PBB treatment. Corticosterone levels in plasma were elevated in treated mice relative to controls, but not elevated enough to be responsible for the immunological findings. No histopathological effects were observed in the thymus, spleen, or lymph nodes of mice treated with 30 mg/kg/day FireMaster BP-6 for 4–5 weeks, but relative thymus weight was temporarily decreased (Gupta et al. 1981). Other studies in mice reported increased lethality ($p<0.05$) after bacterial inoculation in groups treated with 10 mg/kg/day FireMaster FF-1 for 6 months (Luster et al. 1980) and increased lethality ($p<0.05$) due to challenge with *Salmonella thyphosa* lipopolysaccharide after 3 or 6 weeks of dietary exposure to .21.7 mg/kg/day FireMaster FF-1 (Loose et al. 1981). No histopathological changes were observed in the spleen, thymus, and lymph nodes of mice treated with up to 10 mg/kg/day FireMaster FF-1 for 25 weeks (NTP 1983), although 3.9 mg/kg/day for up to 105 weeks caused increased splenic hematopoiesis (NTP 1992).

Guinea pigs administered 0.4 mg/kg/day FireMaster BP-6 in the diet for 45 days exhibited a significant reduction ($p<0.01$) in tetanus-antitoxin titers following injection of tetanus toxoid (Vos and van Genderen 1973, 1974). A dose 5 times higher caused marked thymus atrophy, splenic effects (marked depletion of the follicles and periarteriolar lymphocyte sheaths), and lethality. Pregnant sows fed a diet that provided approximately 2.5 mg/kg/day FireMaster BP-6 for a total of 12 weeks (including part of gestation and lactation) showed a significantly reduced ($p<0.05$) lymphocyte response to stimulation with PHA and PWM mitogens relative to controls (Howard et al. 1980); a dose of 1.25 mg/kg/day was without effect. However, PBB treatment did not affect bactericidal activity of whole blood towards *Escherichia coli* and *Staphylococcus aureus*.

Two cows gavaged with daily doses of 67 mg/kg/day of an unspecified PBB mixture for 38 consecutive days showed minimal alterations in tests of humoral and cell immunity relative to a group of 54 unexposed animals (Kateley et al. 1982). The concentration of PBBs in tissues from these two cows reached 1,000 mg/kg, and they became moribund and were later sacrificed. A similar lack of significant immunological effects was reported in the same study for 58 cows from contaminated farms in Michigan that had PBB body burdens ranging from 0.02 to 24 mg/kg for at least 2 years (Kateley et al. 1982). Cows that received gavage doses of #0.65 mg/kg/day FireMaster PB-6 for 60 days showed no histopathologic alterations in the thymus or spleen (Moorhead et al. 1977). However, doses of 67 mg/kg induced thymic involution and atrophy, and were nearly lethal.

The highest NOAEL values and all LOAEL values from each reliable study for immunological effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

Polybrominated Diphenyl Ethers. No studies were located regarding immunological effects in humans after oral exposure to PBDEs. No effects on pokeweed mitogen-stimulated DNA proliferation or IgG immunoglobulin synthesis were found in human lymphocytes exposed to 2,2',4,4'-tetraBDE or 2,2',3,4,4'-pentaBDE *in vitro* (Fernlof et al. 1997).

Limited information is available on effects of acute-duration exposure to pentaBDE on immunologic function in animals. A single gavage dose of 0.8–500 mg/kg of pentaBDE (DE-71) did not effect the plaque-forming splenic cell (PFC) antibody response to injected SRBC in mice (Fowles et al. 1994). Mice that were given 18, 36, or 72 mg/kg/day doses of pentaBDE (DE-71) by gavage for 14 days had significantly reduced antibody response to SRBC (63% of control value, $p<0.02$) and decreased thymus weight at 72 mg/kg/day (Fowles et al. 1994). There were no exposure-related effects of the 14-day

exposure to #72 mg/kg/day on natural killer cell (NKC) activity to murine YAC-1 target cells; NKC activity was not evaluated in the single dose study. A 14-day study of another pentaBDE mixture (Bromkal 70-5 DE) was conducted in which mice and rats were administered 18 or 36 mg/kg/day by gavage and were evaluated for spleen and thymus weights, numbers of splenic and thymic lymphocyte subsets (CD4+, CD8+, and CD45R+ cells), and *in vitro* IgG immunoglobulin production in pokeweed mitogen-stimulated splenocytes (Darnerud and Thuvander 1998). The only exposure-related effect in either species was significantly reduced *in vitro* production of IgG in pokeweed-stimulated splenocyte cultures from the mice exposed to 36 mg/kg/day. Mice that were similarly tested with 18 mg/kg/day of the congener 2,2',4,4'-tetraBDE for 14 days had significantly reduced numbers of total splenocytes as well as CD4+, CD8+, and CD45R+ cells in spleen (Darnerud and Thuvander 1998). This was the only dose level of a single congener used in the mice. Rats were not evaluated.

Histopathological examinations of spleen, thymus, lymph node and/or bone marrow tissues showed no effects of repeated dietary administration in intermediate-duration studies in rats exposed to #8,000 mg/kg/day decaBDE for 13 weeks (NTP 1986), in mice exposed to #9,500 mg/kg/day decaBDE for 13 weeks, in rats exposed to #750 mg/kg/day octaBDE for 13 weeks (IRDC 1977), or in rats exposed to #100 mg/kg/day pentaBDE for 90 days (WIL Research Laboratories 1984). Chronic ingestion of decaBDE caused lesions in the spleen of rats exposed to $1,200 mg/kg/day (splenic hematopoiesis in males) or 2,240 mg/kg/day (splenic fibrosis and lymphoid hyperplasia in females) for 103 weeks (NTP 1986).

The highest NOAEL values and all LOAEL values from each reliable study for immunological effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.4 Neurological Effects

Polybrominated Biphenyls. Neurological symptoms were reported frequently by Michigan residents during a 3–4-year period following the 1973 PBB contamination episode, but positive associations between serum PBB levels and frequency of neurological symptoms were not found in several studies. In an epidemiological study conducted by the U.S. Center for Disease Control and the Michigan Department of Public Health, fatigue was reported more frequently by several putatively exposed groups including 2,148 residents of farms quarantined for PBB contamination (36.4% prevalence rate), 1,421 recipients of food from contaminated farms (32.4%), 252 chemical workers involved in PBB manufacturing or distribution (22.0%), and 331 residents of farms with low levels of PBB contamination (41.4%), than by a small (60 persons) unexposed control group (15.8%); however, no positive association was apparent between serum levels of PBB and prevalence rates for any reported symptom (Landrigan et al. 1979). Neurological symptoms, including marked tiredness and decrements in the capacity for intellectual and physical work, also were reported with greater frequencies in groups of farmers and residents of Michigan likely to have consumed farm products contaminated with PBB, than in groups of unexposed Wisconsin farmers; however, serum PBB levels were not positively associated with prevalence rates for any symptom including neurological symptoms, nor with performance on neurobehavioral tests for a subset of this population (Anderson et al. 1978c, 1979; Valciukas et al. 1978, 1979). In a 1976 medical history questionnaire study of 342 Michigan children likely to have been exposed to PBBs and 72 unexposed children from Wisconsin, the number of subjectively reported symptoms of ill health, including several symptoms of neurological effects, did not increase with increasing serum PBB levels (assayed in 1976), but rather decreased; general neurological examinations did not reveal a pattern of abnormality among the Michigan children (Barr 1980). Subjectively reported symptoms of neurological effects including weakness, fatigue, difficulty in concentrating, and irritability were prevalent in a group of 23 farmers involved in the Michigan PBB contamination episode, but tests of intelligence, memory, and nerve conduction velocity failed to demonstrate abnormalities. In addition, a group of 28 workers involved in the manufacture or distribution of PBB displayed higher average serum PBB levels than the farmers (48 ppb versus 14 ppb), but did not report a prevalence of symptoms of neurological effects (Stross et al. 1981). In a study of 21 Michigan residents who consumed PBB-contaminated food and had lingering medical complaints and 21 volunteer control subjects with putative low-dose exposure to PBB, no positive association was observed between PBB levels in fat tissue and performance in a battery of neuropsychological tests (Brown and Nixon 1979). In general, the findings of the epidemiological and clinical studies of people exposed to PBBs in Michigan are inconclusive; they do not clearly demonstrate

or eliminate the possibility of an association between PBB oral exposure and the occurrence of neurological effects.

Limited data indicate that orally (gavage) administered PBBs can produce neurological effects in rats. FireMaster FF-1 at 10 mg/kg/day (3 days/week) for 8 weeks did not alter the performance of rats in tests of operant behavior, but decreased motor activity, grip strength, and startle responsiveness observed in rats following administration of #10 mg/kg/day for 6 months or 30 mg/kg/day for 4 weeks (Tilson and Cabe 1979). Motor activity changes were also observed in rats administered doses of FireMaster FF-1 as low as 1 mg/kg/day for 4 weeks (Geller et al. 1979). In this experiment, neither learning nor performance of a simple discrimination task was affected by 1, 3, or 6 mg/kg/day dosage levels, but increased motor activity was observed at 1 mg/kg/day. No changes were apparent at 3 mg/kg/day and decreased motor activity was apparent at 6 mg/kg/day, compared with controls. Weakness of the hind limb was noted in rats treated with 10 mg/kg/day FireMaster FF-1 for 6 months compared with control rats (Cabe and Tilson 1978). Histological examination of brain and/or spinal nerve tissue found no FireMaster FF-1-related alterations in rats or mice administered up to 10 mg/kg/day for 25 weeks (NTP 1983) or 3.9 mg/kg/day for up to 105 weeks (NTP 1992).

Neurodevelopmental effects were assessed in offspring of mice that were treated with 3 or 10 mg/kg/day doses of FireMaster (FF-1) in corn oil by gavage on every other day during gestation and until weaning of the offspring at 21 days of age (Tilson 1992). Acoustic startle response, negative geotaxis, motor activity, and body weight were measured in 8 pups/sex/dose at 30, 60, and 120 days of age. Tests for avoidance learning and neurochemistry were performed on one pup/sex/dose at 30 days of age and on the remaining animals at 120 days of age. Reductions in acoustic startle responsiveness and negative geotaxis latency were observed at 10 and $3 mg/kg/day, respectively, in both sexes at 30 and 60 days of age. Motor activity was decreased in 10 mg/kg/day females at 120 days of age. The learning tests showed increased avoidance response latencies at 30 and 120 days of age in both sexes at $3 mg/kg/day, but no effect on acquisition or retention. Neurochemical measurements included serotonin and metabolites, dopamine and metabolites, and norepinephrine in the cortex, hippocampus, and striation; the only effect observed was a decrease in dopamine concentration in the striation of both males and females at 120 days of age.

Postnatal neurodevelopmental effects were also evaluated in offspring of rats that received 0.2 or 2 mg/kg/day doses of FireMaster BP-6 dissolved in peanut butter from day 6 of gestation through day 24 postpartum and observed until postnatal day 60 (Henck et al. 1994). Multivariate analysis of variance of neurodevelopmental end points showed significant PBB-related effects for acquisition of forward

locomotion, cliff avoidance, cage emergence, and open-field activity in male and female offspring of the rats exposed to 2 mg/kg/day. The most prominent behavioral effects were delays in acquisition of forward locomotion and suppressed open-field activity. Other effects in the offspring included reduced crown-rump length and body weight at birth and reduced postnatal body weight as summarized in Section 3.2.2.6 (Developmental Effects).

The highest NOAEL values and all LOAEL values from each reliable study for neurological effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

Polybrominated Diphenyl Ethers. No studies were located regarding neurological effects in humans after oral exposure to PBDEs.

No information was located on neurological effects of oral exposure to commercial mixtures of PBDEs in animals. Behavioral tests of individual congeners in mice indicate that the developing nervous system is a potential target of PBDE toxicity. Neonatal male NMRI mice were treated with a single dose of 2,2',4,4'-tetraBDE (0.7 or 10.5 mg/kg) or 2,2',4,4',5-pentaBDE (0.8 or 12.0 mg/kg) in fat emulsion by gavage on Pnd 10 (Eriksson et al. 1998, 2001). Spontaneous behavior was tested at 2 and 4 months of age in all dose groups, and learning and memory ability (Morris swim maze performance) was tested at 5 months of age in the groups given the high dose of each congener. Measurements of locomotion, rearing, and total activity during three consecutive 20-minute periods in the spontaneous behavior test showed a disruption of habituation in mice exposed to both congeners. Habituation was defined as a decrease in the activity variables in response to the diminishing novelty of the test chamber over 60 minutes. During the first 20-minute period, at both 2 and 4 months, animals treated with 10.5 mg/kg 2,2',4,4'-tetraBDE and $0.8 mg/kg 2,2',4,4',5-pentaBDE were significantly less active than controls as shown by dose-related decreases in all three test variables. During the second period, the activities in all treated groups were comparable to controls at 2 and 4 months. During the third period, exposure to 10.5 mg/kg 2,2',4,4'-tetraBDE and $0.8 mg/kg 2,2',4,4',5-pentaBDE caused significantly more activity than controls at 2 and 4 months as shown by dose-related increases in test variables. The investigators noted that this nonhabituating behavior profile (i.e., decreased activity early in the test period and increased activity late in the test period) has also been reported in adult mice neonatally exposed to certain PCB congeners. The Morris water maze test evaluated cognitive abilities by providing visual cues to find and remember the location of a platform submerged in a pool of water. The decrease in time needed to locate the platform over a total of 20 trials in a 4-day acquisition period was used as a measure of learning ability. On day 5, the platform was relocated and the decrease in time needed to find the

relocated platform over five trials was used to assess relearning ability. The only exposure-related effect was found in the mice administered a single dose of 12.0 mg/kg 2,2',4,4',5-pentaBDE (that did not improve in finding the new location of the platform as did control animals and those in the 2,2',4,4'-tetraBDE group).

Spontaneous locomotion behavior was also evaluated in adult NMRI mice (4 months old) that were exposed as neonates on Pnds 3, 10, or 19 to a single oral dose of 8 mg/kg of 2,2',4,4'-tetraBDE (Eriksson et al. 1999). A non-habituating behavior profile similar to that observed in the Eriksson et al. (1998, 2001) studies was observed in the mice treated at either 3 or 10 days of age. There was no effect on spontaneous activity in the mice treated at 19 days of age, suggesting that there was a critical window for the induction of behavioral disturbances.

3.2.2.5 Reproductive Effects

Polybrominated Biphenyls. Analysis of semen from 41 male residents of Michigan who lived on PBB-contaminated farms or who had bought food directly from such farms and 11 males who were employed in a PBB manufacturing company revealed no abnormalities in the distribution of sperm counts, sperm motility, or sperm morphology, compared with an analysis of semen from 52 unexposed men (Rosenman et al. 1979). This study was conducted in 1977, .4 years after initial contamination of Michigan's food supply, and would not have detected an earlier response that was subsequently reversed. PBBs were detected (detection limit of 0.2 ppb) in the serum of 1 of the 52 unexposed men and in all of the exposed men; however, individual or mean values for PBB levels were not reported.

No relationship was found between serum levels of PBBs and frequency and duration of lactation in a retrospective study of women exposed to PBBs during the Michigan contamination episode (Thomas et al. 2001). A group of 1,020 women with available initial serum PBB levels was identified from the Michigan Department of Community Health PBB registry. Among these participants, 446 had a live-born infant after their initial serum PBB level; characteristics of this cohort included mean age of 25.6±5.0 years, initial serum PBB level of 17.5±99.7 ppb, estimated serum PBB level at delivery of 9.4±61.9 ppb, estimated serum PCB level at delivery of 5.5±5.2 ppb, duration of breast-feeding as main source of nutrition of 2.6±3.3 months, and total duration of breast feeding of 4.1±5.3 months. The numbers of women who breast fed their first infant after the initial serum PBB level and had previously breast-fed were 293 (65.7%) and 49 (11.0%), respectively. Exposure was treated as a categorical variable by dividing the women into groups of low (reference) exposure (#1 ppb, n=260, serum levels at or below

the detection limit), moderate exposure (>1–#7 ppb, n=141), and high exposure (>7 ppb, n=45, levels above the 90th percentile). Three outcomes of interest were analyzed: (1) the decision to breast feed (yes/no), (2) the duration (months) of breast-feeding as the main source of nutrition, and (3) the total duration (months) of breast-feeding. None of the three outcomes was significantly associated with serum PBB levels, even after controlling for maternal age, previous history of breast-feeding, body mass index, maternal education, household income, history of smoking in the year before pregnancy, consumption of alcohol during the first trimester of pregnancy, history of thyroid disorder, gestational age of the infant, time to pregnancy, and year of birth.

Effects on reproductive organs and reproductive function have been observed in animals following oral exposure to PBBs. An increased incidence of uterine endometrial polyps was observed in rats, 2 years after they were administered a single gavage of 1,000 mg/kg dose FireMaster FF-1 (Kimbrough et al. 1981). Following weaning and two consecutive normal menstrual cycles in 6 months, serum progesterone was decreased in the same four females that showed this effect prebreeding. In a multiple-generation study in which only F_0 rats were fed $5 mg/kg/day FireMaster BP-6 in the diet from Gd 8 through postpartum day 28 (weaning), reproductive performance with respect to length of gestation or litter size was not affected in the F_1 or F_2 generations (McCormack et al. 1981). Implantation was completely blocked in two of five and two of three female rats that survived gavage administration of 28.6 or 57.1 mg/kg/day FireMaster BP-6, respectively, on alternate days between Gds 0 and 14 (Beaudoin 1979). Histological examination of reproductive organs in male and female rats and mice revealed no abnormalities following gavage treatment with doses up to 10 mg/kg/day FireMaster FF-1 for 25 weeks or 30 mg/kg/day for 4–5 weeks (Gupta and Moore 1979; Gupta et al. 1981; NTP 1983). Necrosis, hyperplasia, and metaplasia in the epithelial lining of the ductus deferens were observed in male rats that died following 100 mg/kg/day FireMaster FF-1 for 4–5 weeks (Gupta and Moore 1979). Treatment of male and female mink with diets providing up to 0.39 mg/kg/day FireMaster FF-1 for 6–7 months before breeding did not affect reproductive performance with respect to fertility or litter size (Aulerich and Ringer 1979; Ringer et al. 1981). In the only chronic study, histological examination of male and female reproductive organs showed increased cystic endometrial hyperplasia in rats exposed to 1.5 mg/kg/day FireMaster FF-1 for up to 104 weeks, but no changes were observed in mice exposed to #3.9 mg/kg/day for up to 105 weeks (NTP 1992). Following 6–7 months of exposure to 0.012 mg/kg/day FireMaster FF-1 in the diet, four of seven female monkeys displayed a lengthening of the menstrual cycle from 28 to 31 days and decreased serum progesterone; prior to the treatment, they had at least 2 years of normal cycles (Allen et al. 1979; Lambrecht et al. 1978). All seven of these monkeys conceived after one to four matings with control males (controls required one to three breedings), but two displayed prolonged

implantation bleeding and another two had fetuses that were aborted or stillborn (see Developmental Effects). Reduced spermatogenesis was observed in a male monkey that died after 25 weeks on a diet providing 0.73 mg/kg/day FireMaster FF-1 (Allen et al. 1978).

The highest NOAEL values and all LOAEL values from each reliable study for reproductive effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

Polybrominated Diphenyl Ethers. No studies were located regarding reproductive effects in humans after oral exposure to PBDEs.

Information on effects of PBDEs on reproductive function is limited to negative findings in a one-generation study of decaBDE (FR-300 BA) in rats (Dow Chemical Co. 1975; Norris et al. 1975b). This commercial mixture was comprised of 77.4% decaBDE, 21.8% nonaBDE, and 0.8% octaBDE and differs from current decaBDE formulations that contain $97% decaBDE. Male and female rats were exposed to 3, 30, or 100 mg/kg/day doses in the diet for 60 days before mating and subsequently during a 15-day period during which they were mated. Both sexes continued to receive the test diet throughout gestation and until the end of the 21-day lactation period. Parameters monitored included length of time between first day of cohabitation and parturition, numbers of live and dead newborn, number of live pups (Pnds 1, 7, 14, and 21), litter weight (Pnds 1, 7, and 14), and weanling weight (Pnd 21). Comprehensive histological examinations (adults and weanlings), skeletal examinations (weanlings), and cytogenetic evaluation of bone marrow (adults and weanlings) were also performed on Pnd 21. There were no exposure-related effects on reproductive parameters or any indications of maternal or neonatal toxicity.

No histopathological changes were observed in male or female reproductive tissues from rats that were exposed to decaBDE in dietary doses of #800 mg/kg/day for 30 days (Norris et al. 1973, 1975b), #8,000 mg/kg/day for 13 weeks (NTP 1986), #1.0 mg/kg/day (77.4% containing 21.8% nonaBDE and 0.8% octaDBE) for 2 years (Kociba et al. 1975; Norris et al. 1975b), or #2,550 mg/kg/day for 103 weeks (NTP 1986), or in mice exposed to #9,500 mg/kg/day for 13 weeks or #7,780 mg/kg/day for 103 weeks (NTP 1986); octaBDE in doses of #750 mg/kg/day for 13 weeks (IRDC 1977); or pentaBDE in doses of #100 mg/kg/day for 90 days (WIL Research Laboratories 1984).

The highest NOAEL values and all LOAEL values from each reliable study for reproductive effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.6 Developmental Effects

Polybrominated Biphenyls. Examination of children (. 100 were identified) presumably exposed *in utero* or in early infancy during the peak of the Michigan PBB contamination episode and whose families lived on farms known to be contaminated with PBBs has not revealed any consistent or marked abnormalities in the children's physical and neuropsychological development. No significant abnormalities were found by physical and neurological examination of 33 of these exposed children when they had a mean age of 37.2 months, compared with a group of 20 age-matched, nonexposed control children (Weil et al. 1981). However, subjective interviews with parents suggested that more exposed children than control children had frequent upper respiratory illnesses such as colds, runny noses, and sore throats (Weil et al. 1981). PBBs were measured in the fat of the infants and in the blood of the mothers. Fat levels of PBBs in 27 of the children ranged from 0.01 to 20.96 ppm; half of the values were below 0.120 ppm, and five of the values were above 1.0 ppm. Maternal blood levels ranged from 0.001 to 0.845 ppm and seven mothers had levels that were not detectable (<0.001 ppm). Seagull (1983) administered 5 of 18 tests in a battery of childhood developmental tests (McCarthy Scales of Children's Abilities) to 19 of these exposed children when their ages ranged from . 2.5–4 years old and concluded that there was a statistically significant negative correlation for four of the five tests between PBB levels in fat tissue and developmental abilities. Mean fat concentrations of PBBs were 0.50 ppm (range, 0.10–0.74 ppm) and 4.218 ppm (range, 0.116–20.960 ppm) in the low and high exposure groups of this study. Schwartz and Rae (1983) later administered the full battery of neuropsychological developmental tests, as well as I.Q. tests, to the same group of children (minus one child whose family refused to participate in the follow-up study) when their ages ranged from approximately 4 to 6 years old. The exposed children's performances were within the normal range in all areas assessed. There were statistically significant negative correlations between PBB levels in adipose tissue (measured in the earlier study) and performance on some of the developmental tasks, but the tasks with significant correlations were not the same as those noted in the earlier study by Seagull (1983). The available studies, primarily due to the small data set and the inconsistency of the results, do not conclusively establish or eliminate the possibility that *in utero* and early infancy exposure to PBBs might adversely affect the development of human children. The information suggests that if the Michigan PBB contamination episode produced any effects on child development, they were subtle.

A comparison of 1966–1981 fetal mortality rates for Michigan counties with a high percentage of quarantined farms (6.8–20.4%) with those of Michigan counties with no quarantined farms did not conclusively establish differences in rates or trends after the 1973 contamination episode (Humble and

Speizer 1984). This study is difficult to interpret because the exposure status method was imprecise, the collected data included only spontaneous abortions occurring after 20 weeks of gestation (early trimester abortions were not counted), and the two populations displayed different pre-1973 trends for fetal mortality rates.

Results from animal studies indicate that *in utero* exposure to PBBs and exposure to PBBs through mothers' milk can produce embryolethal effects, structural abnormalities, growth retardation, liver effects, and neurological effects in offspring. Developmental toxicity has been observed in studies with hexabromobiphenyl and octabromobiphenyl commercial mixtures, but not with commercial decabromobiphenyl PBBs. Rats have been studied most extensively, but data are also available for mice, swine, minks, and monkeys.

Following gavage administration of 200 mg/kg FireMaster FF-1 to rats on gestation days 7 and 14, decreased pup survival to weaning, decreased body weight throughout the lives of offspring, and increased mortality in offspring after 2 years were observed (Groce and Kimbrough 1984). Single doses of 200 mg/kg FireMaster BP-6 administered to rats on one of several days during pregnancy caused increased fetal resorptions, and 400 or 800 mg/kg produced maternal toxicity (expressed as a decrease in body weight gain) and fetal malformations including cleft palate and diaphragmatic hernia (Beaudoin 1977). Increased fetal resorptions also were observed in rats receiving total doses of . 14.3 mg/kg/day FireMaster BP-6 by gavage on alternate days from days 0 through 14 of pregnancy (Beaudoin 1979). Body weight gain and levels of vitamin A in the liver were reduced in offspring of rats administered 5 mg/kg/day FireMaster BP-6 in the diet on gestation day 8 until weaning at 4 weeks postpartum (McCormack et al. 1982c). Additional effects in pups weaned onto the same treated diets as the dams included increased hepatic ALA synthetase activity (the rate-limiting enzyme in porphyrin synthesis) and increased urinary excretion of uro- and coproporphyrins at age 16 weeks. Dietary administration of FireMaster BP-6 in dosages of 42.9 mg/kg/day on Gds 7–15 or 50 mg/kg/day on Gds 7–20 produced decreased body weight, but no other developmental effects, in rat fetuses and pups monitored up to 60 days postpartum (Corbett et al. 1975; Harris et al. 1978b). Increased incidences of fetuses with extra ribs were found in rats fed diets providing $86 mg/kg/day of octabromobiphenyl mixture from gestation days 6 through 15 (Waritz et al. 1977); however, no embryotoxic, fetotoxic, or teratogenic effects occurred in rats following gavage administration of #1,000 mg/kg/day decabromobiphenyl mixture on Gds 6–15 (Millischer et al. 1980).

Effects in offspring of rats exposed to 0.5 mg/kg/day FireMaster FF-1 for 60 days before breeding until 8 weeks postpartum (4 weeks postweaning) and observed for up to the following 2 years included vacuolization and altered foci in the liver (Chhabra et al. 1993; NTP 1992). Pups of mice that were similarly perinatally exposed to 1.5 mg/kg/day FireMaster FF-1 developed liver cytomegaly and altered foci (Chhabra et al. 1993; NTP 1992). As discussed in Section 3.2.2.8 (Carcinogenic Effects), these mice also developed hepatocellular adenoma and carcinoma; combined perinatal and adult exposure induced higher incidences of liver tumors in mice than adult exposure alone (Chhabra et al. 1993; NTP 1992).

In a multiple-generation study, decreased pup survival to weaning, decreased body weight gain, delayed fur development, delayed eye and vaginal opening, and increased liver weight associated with hepatocyte swelling, vacuolization, and focal necrosis were observed in F_1 generation rats whose only exposure was from the mothers fed a diet providing 5 mg/kg/day FireMaster FF-1 from day 8 of pregnancy until weaning at 28 days postpartum; less severe liver responses were observed in the F_1 offspring of dams treated with 0.5 mg/kg/day (McCormack et al. 1981). Although survival of F_2 and F_3 generations was not affected by the 5 mg/kg/day treatment of the F_0 rat dams, F_2 offspring, but not F_3 offspring, displayed increased liver weights, liver enzyme induction, and hepatic histological alterations compared with controls (McCormack et al. 1981). Dietary administration of 2.5 mg/kg/day FireMaster BP-6 to rats from gestation day 0 through postpartum day 15 produced increased relative liver weights, decreased body weights, and decreased serum levels of the thyroid hormone, T_4, in 15-day-old offspring (Meserve et al. 1992). The pups had received direct stimulation of the pituitary by an injection of corticotropin-releasing factor or direct stimulation of the adrenals by an injection of adrenocorticotropic hormone. Provision of a diet containing 0.5 mg/kg/day FireMaster FF-1 to lactating rats for the 18 days following parturition increased liver weights and elevated levels of hepatic cytochrome P-450 and associated enzymic activities in both dams and pups; a diet providing 0.05 mg/kg/day produced no hepatic effects in dams, but induced hepatic enzymes in the pups (Moore et al. 1978). According to the investigators (Moore et al. 1978), the results could indicate that nursing pups are more sensitive than their dams to liver enzyme induction, or that due to the different kinetic parameters among the PBB congeners, the pups received a more potent PBB mixture than the dams. Yet, a third possibility is that the suckling pups received a higher dose of PBBs relative to their body weights due to bioconcentration of PBBs in breast milk (Dent 1978). Performance deficits in tests of operant behavior were observed in the 6-month-old offspring of rat dams given gavage doses of 0.2 or 2 mg/kg/day FireMaster BP-6 from day 6 of gestation until weaning (Henck and Rech 1986), but not in 75-day-old offspring of rat dams given gavage doses of 0.5 or 5 mg/kg/day for 4 weeks prior to mating (Geller et al. 1985). Effects found in offspring of rats exposed to 0.2 or 2 mg/kg/day doses of FireMaster BP-6 in the diet from day 6 of gestation through day 24 postpartum, and

observed through postnatal day 60, included reduced crown-rump length at birth at $0.2 mg/kg/day, reduced birth weight and postnatal body weight gain at 2 mg/kg/day, and supressed acquisition of forward locomotion, cliff avoidance, cage emergence, and open-field activity at 2 mg/kg/day (Henck et al. 1994).

Dietary administration of 50 mg/kg/day FireMaster BP-6 to mice from gestation days 7 through 18 produced decreased fetal body weight and fetal abnormalities including exencephaly, cleft palate, and hydronephrosis; 5 mg/kg/day did not produce significant developmental effects in this study (Corbett et al. 1975). Early postnatal deaths occurred among offspring of mice given 10 mg/kg/day FireMaster FF-1 on alternate days from gestation day 0 until litters were weaned (Luster et al. 1980). Immunological parameters were unaffected in surviving offspring whose mothers received up to 10 mg/kg/day doses, but decreased hematocrit levels were measured in offspring of mothers receiving doses $3 mg/kg/day (Luster et al. 1980). Performance deficits in tests of learning behavior were measured in offspring of female mice that received gavage doses of 3 or 10 mg/kg/day FireMaster FF-1 on alternate days from gestation day 0 through weaning at 21 days of age (Tilson 1992).

Decreased body weight at birth and at 4 weeks after birth were measured in mink kits whose parents were fed diets containing 0.155 mg/kg/day FireMaster FF-1 from 7–8 months prior to mating through 4 weeks postpartum (Aulerich and Ringer 1979; Ringer et al. 1981). Increased relative liver weight, fatty and necrotic hepatic changes, slight hyperplasia in the thyroid, and decreased serum levels of thyroid T_3 and T_4 hormones were observed in 4-week-old offspring of swine fed 2.5 mg/kg/day FireMaster BP-6 in the diet during the second half of gestation and during lactation; 1.25 mg/kg/day produced similar effects on the thyroid, but no necrosis in the liver of 4-week-old nursing pigs (Werner and Sleight 1981). Examination of several parameters of immune function in 4-week-old offspring of sows fed #2.5 mg/kg/day FireMaster BP-6 during gestation and lactation provided no conclusive evidence for immunosuppressive effects (Howard et al. 1980). An abortion and a stillbirth occurred among seven female monkeys that were fed 0.012 mg/kg/day FireMaster FF-1 in the diet for 7 months prior to conception and during pregnancy (Allen et al. 1979; Lambrecht et al. 1978). The surviving five infants had reduced birth weight and postnatal body weight gain, but no gross abnormalities. The incidence of dystocia (difficult birthing) was 50% increased among first- and second-generation offspring of cows treated with 0.65 mg/kg/day FireMaster BP-6 by gelatin capsule for 180 or 202 days during late pregnancy (Willett et al. 1982). The same dosage for 60 days caused a 21.6% increased incidence of dystocia, but this increase was not statistically significant ($p=0.08$). Stillbirths and preweaning deaths were not significantly increased, but all mortality was attributable to dystocia. Incidences of dystocia and calf mortality appeared to be related to higher birth weight, which in turn were correlated with

concentrations of PBBs in maternal blood and tissues. Growth and development were not affected in the surviving calves. Of six pregnant cows that were similarly treated with a maternolethal dosage (67 mg/kg/day) of FireMaster BP-6, three aborted after 30–38 days, and three retained dead fetuses (Moorhead et al. 1977).

The highest NOAEL values and all LOAEL values from each reliable study for developmental effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

Polybrominated Diphenyl Ethers. No studies were located regarding developmental effects in humans after oral exposure to PBDEs.

Information on the developmental toxicity of PBDEs is available from studies of commerical mixtures of deca-, octa- and pentaBDE (Argus Research Laboratories 1985b; Breslin et al. 1989; Dow Chemical Co. 1975, 1985; Life Science Research Israel Ltd. 1987; Norris et al. 1975b; WIL Research Laboratories 1986). None of the BDE mixtures have been shown to be teratogenic in animals. Data on decaBDE indicate that it is fetotoxic in rats at high dose levels that are not maternally toxic. Developmental effects of decaBDE were investigated in rats that were exposed to doses of 10, 100, or 1,000 mg/kg/day by gavage on Gds 6–15 and examined on Gd 21 (Dow Chemical Co. 1985; Norris et al. 1975b). No treatment-related maternal toxicity was observed. The numbers of fetuses with subcutaneous edema and delayed ossification of normally developed skull bones were significantly increased at 1,000 mg/kg/day. Resorptions were significantly ($p<0.05$) increased at $10 mg/kg/day compared to controls as indicated by resorption/implantation site percentages [1% (3/288), 9% (12/141), 10% (13/135), and 4% (9/203)] and percentages of litters with resorptions [12% (3/25), 64% (9/14), 57% (8/14), and 39% (7/18)]. The resorptions were considered secondary to unusually low control values and unrelated to treatment because (1) the data do not follow a dose-response relationship across the three dose levels, and (2) the rates in the high dose group are comparable to historical control values. As discussed in Section 3.2.2.5 (Reproductive Effects), a one-generation study of decaBDE in rats found no effects of parental exposure to #100 mg/kg/day from 60 days before mating through the end of the lactation on numbers of live pups at birth and during the lactation period, body weights of pups at birth or weaning, or skeletal development or soft-tissue histology of pups at weaning (Dow Chemical Co. 1975; Norris et al. 1975b).

OctaBDE commercial mixtures caused skeletal ossification variations in rats and rabbits at maternally toxic levels and other indications of fetotoxicity at lower doses (Argus Research Laboratories 1985b; Life Science Research Israel Ltd. 1987). No maternal effects were observed in rats that were administered 2.5,

10, or 25 mg/kg/day doses of octaBDE (FR-1208) by gavage on Gds 6–15 (Life Science Research Israel Ltd. 1987). Fetal death, as measured by post-implantation loss, was somewhat elevated among litters from $10 mg/kg/day dams in comparison to controls. Post-implantation loss was significantly ($p<0.001$) higher than controls in both the 10 and 25 mg/kg/day groups when Freeman-Tukey arcsine transformed values were tested as normally distributing data (Student's t-test). The toxicological significance of this finding was unclear since under non-parametric assumptions (Mann-Whitney U-test), neither group was significantly different from controls, and post-implantation loss for all treated groups was within the range of laboratory historical control values. There were no indications of skeletal malformations of variations or delayed or retarded ossification in any treated group. Rats that were treated with 2.5, 10, or 25 mg/kg/day doses of a different octaBDE mixture (Saytex 111) by gavage on Gds 6–15 had significantly (p#0.01) increased resorptions (embryo/fetal deaths) at 25 mg/kg/day as shown by nonparametric analysis (Kruskal-Wallis test with Dunn's method of multiple comparisons) (Argus Research Laboratories 1985b). There was an average of 0.6, 1.2, 0.8, and 4.9 resorptions and 14.6, 14.7, 14.0, and 8.0% live fetuses per litter in the control to high dose groups. Average fetal body weights were also significantly reduced at 25 mg/kg/day.

Developmental toxicity testing of another octaBDE mixture (DE-79) in rats used gavage doses of 2.5, 10, 15, 25, or 50 mg/kg/day on Gds 6–15 (WIL Research Laboratories 1986). No exposure-related maternal or developmental effects were observed at #15 mg/kg/day. The only statistically significant ($p<0.05$) finding at 25 mg/kg/day was an increased serum bromide level. Effects observed at 50 mg/kg/day included significantly reduced mean maternal body weight gain during the posttreatment period (Gds 16–20) and fetotoxicity as indicated by increased postimplantation loss due to late resorptions (not significantly increased compared to control group but exceeded historical control range), 39% reduced mean fetal weight ($p<0.01$), skeletal variations (e.g., reduced ossification of the skull and various unossified bones) that were associated with the reduced fetal weights in this group, and single instances of malformations (fetal anasarca, bent limb bones, unilateral absence of 13th rib) commonly associated with maternal toxicity.

The developmental toxicity of a fourth octaBDE technical mixture (Saytex 111) was tested in rabbits exposed to doses of 2, 5, or 15 mg/kg/day on Gds 7–19 and examined on Gd 28 (Breslin et al. 1989). The 15 mg/kg/day group showed evidence of slight maternal toxicity as indicated by decreased body weight gain during Gds 7–20 and 7–28 (not statistically identified), reduced body weight on Gd 28 (7% less than controls, p#0.05), and significantly increased absolute and relative liver weights on Gd 28. Slight

fetotoxicity accompanied the maternal toxicity at 15 mg/kg/day as indicated by a significantly (p#0.05) increased incidence of delayed ossification of the sternebrae.

A developmental toxicity study of pentaBDE (Saytex 115) is available in which rats were administered 10, 100, or 200 mg/kg/day doses by gavage on Gds 6–15 and examined on Gd 20 (Argus Research Laboratories 1985a). The only exposure-related maternal effect was significantly (p#0.01) reduced body weight gain at $100 mg/kg/day during the dosing period. This effect was dose-dependent and increased in severity with continued dosing but recovered during the postdosage period when differences between exposed and control groups were no longer significant. Maternal body weight gain was 2.8, 20.1, and 29.9% less than controls during Gds 6–16 at 10, 100, and 200 mg/kg/day, respectively. There were no exposure-related indications of developmental toxicity. Average fetal body weight per litter was slightly but not significantly reduced at 200 mg/kg/day (2.6% less than controls, p>0.05).

Developmental toxicity was also evaluated in rats that were treated with 1, 10, or 30 mg/kg/day of pentaBDE (DE-71) in corn oil by gavage from Gd 6 through Pnd 21 (Zhou et al. 2002). Dams were sacrificed on Gd 20 and Pnd 22 and offspring were sacrificed on Gd 20 and Pnds 4, 14, 36, and 90. There were no exposure-related effects on maternal body weight gain, litter size, sex ratio, or offspring viability and growth as assessed by numbers of pups at birth and on Pnds 4–21, body weight of pups on Pnds 4–90, and eye opening status on Pnds 11–18. Serum measurements of thyroid T_3 and T_4 hormone levels showed that serum T_4 was significantly reduced in dams at 30 mg/kg/day (Gd 20 and Pnd 22) and offspring at $10 mg/kg/day (Gd 20 and Pnds 4 and 14) as detailed in Endocrine Effects in Section 3.2.2.2.

The highest NOAEL values and all LOAEL values from each reliable study for developmental effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.7 Cancer

Polybrominated Biphenyls. No epidemiological studies were located that provided evidence for an association between exposure to PBBs and the occurrence of cancer in humans, although one case report is available concerning gastrointestinal cancer in a Michigan dairy farmer with known exposure to PBBs and other chemicals.

The Michigan Department of Public Health, the U.S. Center for Disease Control, the National Institutes of Health, the Food and Drug Administration, and the EPA established a cohort of Michigan residents with varying levels of PBB exposure to determine the short- and long-term effects (especially cancer) of exposure to PBBs (Landrigan et al. 1979). The epidemiological and clinical data collected during the first 4 years after the Michigan PBB contamination episode indicated that cancer was not a prevalent "symptom" among the cohort at that time. Prevalence rates for cancer in exposed groups ranged from 0.4 to 0.6% compared with 0% in a small control group comprised of residents of farms with low PBB contamination (Landrigan et al. 1979). When the cohort was divided into seven groups based on serum PBB levels, no trend with concentration was apparent, but the incidence of cancer was the highest in the group with the highest serum PBB levels. Subsequent follow-up examinations of this cohort have not been reported.

In studies conducted by the Environmental Science Laboratory of the Mount Sinai School of Medicine, mean plasma levels of carcinoembryonic antigen (CEA), which has been used as a screening tool for tumor recurrence after cancer therapy, were found to be slightly higher in 1976 in a population of 611 Michigan residents who likely ingested PBB-contaminated food than mean levels in a nonexposed population of Wisconsin farm residents, but the difference was not statistically significant (Anderson et al. 1978b). Cancer was not listed as a condition in the report of results of a symptomatology survey completed by this cohort (Anderson et al. 1979). Reports of follow-up examinations of this cohort have not been reported.

A relationship between serum PBBs and risk of breast cancer was suggested in a nested case-control study of 1,925 women enrolled in the Michigan Department of Public Health registry for persons exposed to PBBs (Henderson et al. 1995). Study participants had lived on or received food from a farm quarantined by the Michigan Department of Agriculture, were recruited from July 1976 to December 1977, and followed up annually from 1978 through 1993. Twenty women who developed breast cancer were age- and race-matched to 290 controls. Median serum PBB concentrations were similar in the

cancer cases (3 ppb, range 0.5–16 ppb) and controls (2 ppb, range 0.5–419 ppb). Conditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for increasing serum PBB levels categorized into tertiles (<2 ppb, 2–3 ppb, $4 ppb) and a dichotomous variable (<2 ppb, $2 ppb). The estimated risk for breast cancer was slightly elevated for women with serum PBB levels of 2–3 ppb (OR=3.5, 95% CI=0.9–13) and $4 ppb (OR=3.1, 95% CI=0.8–12) when compared with the reference group (<2 ppb), or when the dichotomous variable was used in the analysis (OR=3.3, 95% CI=0.9-11.4). The results were essentially the same when the data were adjusted for available risk factors (body mass index and family history of cancer), or when matched sets of cases and controls were stratified into two groups based on date of diagnosis ($10 years after exposure). The results of this study are inconclusive due to the small number of cases, apparent lack of statistically significant increases (p values were not reported, but confidence intervals were broad with lower limits less than unity, indicating that it is difficult to exclude chance as an explanation for the findings), and lack of information on important breast cancer risk factors (e.g., exposure to other organochlorine chemicals and estrogen receptor status).

Another study of the Michigan PBB registry evaluated associations between levels of serum PBBs and risks of various site-specific cancers (Hoque et al. 1998). Primary cancers (195 malignancies) were identified in 187 persons among 3,899 registrants enrolled in 1976 and followed through 1993. Controls were 696 randomly selected cancer-free individuals who were frequency matched to cases by age (in 5-year strata) and sex in a 4:1 ratio (except above age 70 years when, due to lower numbers, all eligible controls were used). PBB levels in the cases were measured at the time of registry enrollment. Serum PBB concentration ranges was categorized into four groups (not detectable–3 ppb, 4–20 ppb, 21–50 ppb, >50 ppb) defined by the median and the 90th and 95th percentiles. Conditional logistic regression was used to calculate univariate and multivariate (adjusted) ORs by cancer site for the three highest serum PBB categories compared to the reference (#3 ppb) group. The multivariate ORs were adjusted for family history of cancer, smoking status, alcohol use, age, serum PCB level, and sex. Digestive system cancer (12 cases) and lymphoma (not otherwise specified) (8 cases) showed increasing dose-response relationships for risk as PBB concentrations increased. Digestive system cancer was a grouping that comprised of the following sites: liver (five cases), stomach (five cases), esophagus (one case) and pancreas (one case). Adjusted ORs for digestive system cancer were 1.00 (reference), 8.23 (95% CI=1.27–53.3), 12.3 (0.80–191), and 22.9 (1.34–392) for the #3, 4–20, 21–50, and >50 ppb categories, respectively. The corresponding adjusted ORs for PBB level and lymphoma risk were 1.00, 3.85 (0.32–46.2), 19.6 (1.52–253), and 48.9 (4.09–585). The lymphoma ORs were incompletely adjusted due to missing data for serum PCB level and family cancer history in the reference category. Increased

risks were also observed for breast cancer in the 4–20 ppb category (nine cases, adjusted OR=2.41, 95% CI=0.92–6.30), cancer at an unknown site in the 4–20 ppb category (four cases, adjusted OR=31.0, 95% CI=1.40–685), and leukemia in the 21–50 ppb category (one case, adjusted OR=4.49, 95% CI=0.92–6.30). The associations found in this study should be viewed as suggestive and preliminary due to the small numbers of cases. The 2.4-fold increased risk of breast cancer for PBB levels between 4 and 20 ppb is consistent with the 3-fold increased risk for breast cancer observed for PBB levels >2 ppb in the Henderson et al. (1995) study of the same cohort summarized above.

A Michigan dairy farmer, who had a history of health complaints after 1976, developed malignant cancer of the esophageal and stomach wall in 1986; the man subsequently died in 1988 (Sherman 1991). Samples of adipose tissue, collected in 1976 and 1987, revealed PBB concentrations of 0.83 and 0.85 ppm, respectively. Also detected in the fat tissue collected in 1987 were polychlorinated biphenyl (PCB) at 3.57 ppm and chlordane residues at concentrations ranging from 0.018 to 0.039 ppm.

FireMaster FF-1 has induced liver tumors and/or neoplastic nodules in rats and mice following single or repeated administration by gavage in oil vehicle, as well as following chronic dietary administration. In female Sherman rats given a single 1,000 mg/kg dose and observed for 2 years, incidences of hepatocellular carcinomas and liver neoplastic nodules were 41.4% (24/58) and 72.4% (42/58), respectively (Kimbrough et al. 1981). In an earlier study using the same treatment (single 1,000 mg/kg dose), groups of five Sherman rats of each sex were examined at 2, 6, 10, and 14 months following treatment (Kimbrough et al. 1978b). Neoplastic nodules were found in the livers of 22.5% (9/40) of the treated rats observed for at least 10 months (4/5, 2/5, and 3/5 in the 10-month females, 14-month males, and 14-month females, respectively). Liver tumors were not found, but this could be related to the relatively small number of animals (20/sex) and/or short duration of observation (#14 months). Liver neoplastic nodules without tumors also developed in 31.2% (5/16) of Sherman rats treated with a lower single dose (200 mg/kg) and observed for 18–22 months (Kimbrough et al. 1981). No liver tumors or neoplastic nodules developed in untreated control groups in any of these single dose studies, and treatment-related tumors in sites other than liver were not observed. When administered to pregnant rats once on gestation day 7, a dose of 200 mg/kg induced both hepatocellular carcinomas and liver neoplastic nodules in offspring that were observed for 2 years (Groce and Kimbrough 1984). The incidences of tumors in male offspring (9.6% [4/41] versus 0% [0/42] in controls) and nodules in female offspring (17.6% [9/51) versus 4.2% [2/48]) increased significantly (p#0.055). Treatment-related tumors were not observed in nonhepatic tissues of the offspring.

Hepatocellular carcinomas and liver neoplastic nodules also increased in female Sherman rats gavaged with 100 mg/kg FireMaster FF-1 twice a week for two 3-week periods (12 total doses) separated by .10 weeks (Kimbrough et al. 1981). Following observation for 2 years, the incidence of carcinomas was 60.7% (17/28 versus 0/25 in controls) and incidence of nodules was 85.7% (24/28 versus 1/25). In repeated dose studies performed by NTP (1983), FireMaster FF-1 was administered via gavage to Fischer-344/N rats and B6C3F1 mice of both sexes at dosages of 0, 0.1, 0.3, 1, 3, or 10 mg/kg/day on 5 consecutive days per week for 25 weeks. Both rats and mice were observed for life (up to 23 and 24 months posttreatment, respectively). Incidences of hepatocellular carcinoma were dose-related and significantly ($p<0.01$) increased in male rats at $3 mg/kg/day (0/33 [controls], 2/39, 0/40, 1/33, 7/33, and 7/31 [high-dose]) and female rats at 10 mg/kg/day (7/20 versus 0/20 in controls). The incidence of cholangiocarcinoma was significantly ($p<0.01$) increased in female rats at 10 mg/kg/day (7/20 versus 0/20) and almost significant ($p=0.06$) in males at 10 mg/kg/day (2/31 versus 0/33). Liver neoplastic nodules were dose-related and significantly ($p<0.01$) increased in female rats at $3 mg/kg/day. No clear treatment-related effects on incidences of hepatic neoplastic nodules in males, or bile duct hyperplasia, myelomonocytic (mononuclear cell) leukemia, or foci of pancreas-like tissue in the liver in either sex were observed. In mice, incidences of hepatocellular carcinoma significantly ($p<0.01$) increased at 10 mg/kg/day in males (21/22 versus 12/25 in controls) and females (7/8 versus 0/13). Metastasis to lung also significantly ($p<0.05$) increased in female mice at 10 mg/kg/day. No treatment-related effects on hepatocellular adenomas or hepatoblastomas were observed. Thyroid follicular cell adenoma tended to increase in treated female mice, but data are inconclusive due to low incidences and small numbers of animals.

The carcinogenicity of FireMaster FF-1 was additionally evaluated in Fischer-344/N rats and B6C3F1 mice of both sexes that received adult exposure only, perinatal exposure only, or combined perinatal and adult exposure (NTP 1992). The adult-only exposure involved dietary administration of PBBs (F_1 diets) to .8-week-old animals for up to 104 weeks (rats) or 105 weeks (mice). Perinatal-only exposure involved dietary treatment of dams (F_0 diets) for 60 days prior to breeding and throughout gestation and lactation until pups were 8 weeks old. The pups were administered the same treatment as the dams from weaning at week 4 until age 8 weeks, and were subsequently administered the same or different dietary treatments (F_1 diets) for up to 104 weeks (rats) or 105 weeks (mice). This study was designed to compare the carcinogenicity of PBBs given in a conventional bioassay protocol (i.e., the adult-only exposure) with that of PBBs given in a combined perinatal and adult exposure regimen.

Eight F_0:F_1 doses (estimated) were tested in rats among one unexposed control group (0:0 mg/kg/day), two adult-only exposure groups (0:0.5 and 0:1.5 mg/kg/day), one perinatal-only exposure group (0.5:0 mg/kg/day), and four combined perinatal and adult exposure groups (0.05:0.15, 0.15:0.5, 0.5:0.5, and 0.5:1.5 mg/kg/day) (Chhabra et al. 1993; NTP 1992). Incidences of hepatocellular tumors were increased in adult-only exposed rats of both sexes. In males ingesting 0:0, 0:0.5, and 0:1.5 mg/kg/day, incidences of adenoma were 1 of 50, 10 of 49, and 38 of 50; of carcinoma, 0 of 50, 2 of 49, and 19 of 50; and of adenoma or carcinoma (combined), 1 of 50, 12 of 49, and 41 of 50, respectively. In females, incidences of adenoma were 0 of 50, 10 of 50, and 38 of 50; of carcinoma, 0 of 50, 2 of 50, and 4 of 50; and of adenoma or carcinoma (combined), 0 of 50, 12 of 50, and 39 of 50, respectively. These increases in liver tumor incidences were statistically significant (p#0.002) except for carcinoma in 0:0.5 mg/kg/day males and females and 0:1.5 mg/kg/day females (p>0.05). Combined perinatal and adult exposure significantly enhanced the development of liver tumors in female rats, as shown by comparisons with females receiving adult exposure only. Compared to the 0:0.5 mg/kg/day female adult-only exposed group, incidences of hepatocellular adenoma were 22 of 50 (p=0.01) and 35 of 50 (p<0.001); of carcinoma, 1 of 50 (p=0.05) and 8 of 50 (p=0.048); and of hepatocellular adenoma or carcinoma (combined), 22 of 50 (p=0.03) and 39 of 50 (p<0.001) in the 0.15:0.5 and 0.5:0.5 mg/kg/day groups, respectively. Compared to the 0:1.5 mg/kg/day female adult-only exposed group, incidences of hepatocellular adenoma, carcinoma, and hepatocellular adenoma or carcinoma (combined) were 45 of 50 (p=0.049), 22 of 50 (p<0.001), and 47 of 50 (p=0.016), respectively, in the 0.5:1.5 mg/kg/day group. This enhancing influence of perinatal exposure did not occur in the males. Perinatal-only exposure did not cause significantly increased incidences of liver or other tumors in rats of either sex.

Increased incidences of mononuclear cell leukemia occurred in adult-only exposed rats but were not clearly related to treatment (Chhabra et al. 1993; NTP 1992). The incidences of this leukemia in the 0:0, 0:0.5, and 0:1.5 mg/kg/day groups were 25 of 50, 33 of 50, and 31 of 50, respectively, in males and 14 of 50, 22 of 50, and 23 of 50, respectively, in females; the incidences in the 0:0.5 mg/kg/day males and 0:1.5 mg/kg/day females were significantly (p#0.05) increased. Comparison of the combined perinatal and adult exposure groups with the adult-only exposed groups showed no significant enhancement; however, comparison with the unexposed control (0:0 mg/kg/day) incidences showed a consistent increase in the incidence of this neoplasm at higher doses. In the 0.15:0.5 and 0.5:0.5 mg/kg/day groups, the incidences of leukemia were 41 of 50 (p#0.01) and 37 of 50 (p#0.01) in males and 17 of 50 (p>0.05) and 27 of 50 (p#0.01) in females. In the 0.5:1.5 mg/kg/day groups, the incidences were 37 of 50 (p#0.01) in males and 25 of 50 (p#0.05) in females. The incidences in some of these groups fall outside the NTP historical control range. The incidences in males were as high as 82% and exceeded the upper historical

control range of 62%. In females, the incidences were as high as 54% and exceeded the overall upper historical control range of 52% and the laboratory upper historical control range of 28%. A combined (life table) analysis of data from all eight experimental groups indicates that significant increases in the incidence of the leukemia are associated with increasing F_1 concentrations (p#0.05 in males; p#0.01 in females). For males, there was also a marginally significant (p#0.05) increase associated with F_0 exposure.

Eight F_0:F_1 doses were also tested in the mice among one unexposed control group (0:0 mg/kg/day), two adult-only exposure groups (0:1.3 and 0:3.9 mg/kg/day), one perinatal-only exposure group (3.9:0 mg/kg/day), and four combined perinatal and adult exposure groups (0.39:0.39, 1.3:1.3, 3.9:1.3, and 3.9:3.9 mg/kg/day) (NTP 1992). As in the rats, hepatocellular tumors were significantly ($p<0.001$) increased in adult-only exposed mice of both sexes. In males ingesting 0:0, 0:1.3, and 0:3.9 mg/kg/day, incidences of adenoma were 9 of 50, 48 of 49, and 42 of 50; of carcinoma, 8 of 50, 30 of 49, and 36 of 50; and of adenoma or carcinoma (combined), 16 of 50, 48 of 49, and 48 of 50, respectively. In adult-only exposed females, incidences of adenoma were 4 of 50, 39 of 50, and 46 of 48, carcinoma were 1 of 50, 28 of 50, and 41 of 48, and adenoma or carcinoma (combined) were 5 of 50, 42 of 50, and 47 of 48, respectively. Combined perinatal and adult exposure resulted in increased incidences of liver neoplasms in some treated groups. However, because adult-only exposure to 1.3 or 3.9 mg/kg/day resulted in such high incidences of liver neoplasms (84–98%), the possible enhancing effect of combined perinatal and adult exposure could not be adequately assessed in either sex. Compared to 0:1.3 mg/kg/day adult-only exposure, combined perinatal and adult exposure to 3.9:1.3 mg/kg/day caused significantly increased incidences of carcinoma in males (40 of 50, $p=0.01$) and females (44 of 50; $p<0.001$), adenoma in females (47 of 50, $p=0.005$) and adenoma or carcinoma (combined) in females (50 of 50, $p<0.001$). Compared to 0:3.9 mg/kg/day adult-only exposure, combined perinatal and adult exposure to 3.9:3.9 mg/kg/day caused significantly increased adenoma incidence in males (48 of 50; $p=0.007$) and decreased adenoma incidence in females (41 of 47; $p=0.022$). Perinatal-only exposure also caused significantly increased incidences of liver neoplasms in mice of both sexes. Comparison of the 0:0 and 3.0:0 mg/kg/day groups showed hepatocellular adenoma, carcinoma, and adenoma or carcinoma (combined) incidences of 31 of 50 ($p<0.001$), 17 of 50 ($p=0.033$), and 40 of 50 ($p<0.001$) in males and 19 of 50 ($p<0.001$), 7 of 50 ($p=0.213$), and 21 of 50 ($p<0.001$) in females. Combined perinatal and adult exposure to 3.9:1.3 mg/kg/day also caused a significant ($p=0.029$) increase in the incidence of thyroid follicular cell adenoma in male mice (5 of 48) compared to adult-only exposure to 0:1.3 mg/kg/day (0 of 49). This incidence of thyroid adenoma exceeds the historical control range of 0–4% in untreated males in NTP

studies, but the effect was not seen in the higher dose groups (0:3.9 or 3.9:3.9 mg/kg/day). Perinatal-only exposure did not induce thyroid or other nonhepatic tumors in mice of either sex.

The existing evidence conclusively demonstrates that the liver is the main target of PBB carcinogenicity in animals. Results of a chronic study (Chhabra et al. 1993; NTP 1992) suggest that male rats are more sensitive than female rats (based on a higher carcinoma/adenoma ratio), and that mice are more sensitive than rats (based on earlier occurrence of hepatocellular adenomas, higher combined incidence of all liver neoplasms, and higher liver concentrations of PBBs). Based on findings in male rats and mice of both sexes in this study, there is some evidence that combined perinatal and adult dietary exposure to FireMaster FF-1 enhanced the susceptibility of hepatocellular neoplasms in animals receiving adult exposure.

The Cancer Effect Levels (CELs) for FireMaster FF-1 reported in Kimbrough et al. (1981), Groce and Kimbrough (1984), and NTP (1983, 1992) are recorded in Table 3-2 and plotted in Figure 3-2.

There are data indicating that FireMaster BP-6 has tumor promoting activity in rats and hamsters. In standardized liver tumor promotion assays, development of enzyme-altered hepatic foci (putative preneoplastic lesions) was assessed in rats that were 70% hepatectomized, initiated with a subcarcinogenic intraperitoneal dose of diethylnitrosamine 24 hours after the partial hepatectomy, and promoted with orally administered FireMaster BP-6 beginning 30 days later. Various promotion protocols caused significantly increased numbers of enzyme-altered hepatic foci with gamma-glutamyl transpeptidase (GGT) activity, including two gavage doses of 65 mg/kg on adjacent days (6.5 mg/kg was not effective) (Rezabek et al. 1987), estimated dietary dosages of 0.5 or 5 mg/kg for 180 days (Jensen et al. 1982), and estimated dietary dosages of 0.5 mg/kg/day for 140 days or 5 mg/kg for 15 days (Jensen et al. 1984). In a similar assay with rats that were not hepatectomized, a single gavage dose of 100 mg/kg FireMaster BP-6 administered 7–10 days after initiation with dimethylnitrosamine (NDMA) or N-nitrosopyrrolidine (NPYR) promoted development of hepatic enzyme-altered foci (Rangga-Tabbu and Sleight 1992). A statistically significant increased number of tracheal papillomas (but not number of animals with papillomas) developed in a group of hamsters given a single subcutaneous initiating dose of diethylnitrosamine and fed an estimated dietary dosage of 8.3 mg/kg/day FireMaster BP-6 for 140 days (Wasito and Sleight 1989).

Individual PBB congeners have been examined for tumor promoting activity in rats that were partially hepatectomized and initiated with diethylnitrosamine (DENA). Numbers of hepatic GGT-altered foci

and/or neoplastic nodules were increased following promotion with 3,3',4,4'-tetrabromobiphenyl (. 0.25 mg/kg/day in the diet for 180 days or 8 weekly intraperitoneal injections of . 7 mg/kg), 2,2',4,4',5,5'-hexabromobiphenyl (. 0.5 mg/kg/day in the diet for 180 days) or 3,3',4,4',5,5'-hexa-bromobiphenyl (. 0.05 mg/kg/day in the diet for 140 days) (Buchmann et al. 1991; Dixon et al. 1988; Jensen et al. 1982, 1983). Dietary exposure to . 5 mg/kg/day of 2,2',4,4',5,5'-hexabromobiphenyl for 480 days similarly promoted hepatic development of altered foci and neoplastic nodules in rats, whereas . 0.05 mg/kg/day of 3,3',4,4',5,5'-hexabromobiphenyl did not, although an apparent synergistic effect was observed when these two congeners were fed together (Jensen and Sleight 1986). Additional information on structure-promotion relationships for PBBs is discussed in Section 3.5.2.

The tumor initiating potential of PBBs is not well characterized. Numbers of GGT-altered foci were significantly increased in partially hepatectomized rats that were administered a single 1–10 mg/kg oral dose of 3,3',4,4'-tetrabromobiphenyl followed by phenobarbital in the diet for 180 days (Dixon et al. 1988), indicating that PBBs may have initiating activity in hepatocarcinogenesis. The potential for liver tumor initiation by PBBs appears to be weak compared to their potent promoting activity (Buchmann et al. 1991; Dixon et al. 1988; Jensen et al. 1984).

Polybrominated Diphenyl Ethers. There was no clear association between risk of non-Hodgkin's lymphoma (NHL) and exposure to 2,2'4,4'-tetraBDE in a case-control study of 77 Swedish men and women who were recruited in 1995–1997 and ranged in age from 28 to 85 years (Hardell et al. 1998; Lindstrom et al. 1998). Adipose tissue levels of 2,2'4,4'-tetraBDE (used as a marker for total PBDE exposure) were compared in 19 patients with NHL, 23 patients with malignant melanoma, 8 patients with other cancers or *in situ* changes, and 27 persons with no cancer diagnosis. The highest concentrations were seen in the patients with NHL. The mean concentration of 2,2'4,4'-tetraBDE was 13.0 ng/g (ppb) lipid (range 1.0–98.2 ppb) in the 19 NHL patients and 5.1 ppb (range 0.6–27.5 ppb) in the 27 persons without known malignancies. Logistic regression, adjusted for age, gender, sum of PCBs, and sum of chlordanes, was performed on cases and controls in three concentration groups (<2.05, 2.05–<5.43, and $5.43 ppb). A nonsignificantly elevated risk with a suggestive dose-response was found for NHL in the two highest concentration groups compared with the lowest group; the ORs and 95% CIs were 1.9 (0.3–14) and 3.8 (0.7–26) in the middle and high groups, respectively. Although the risk was highest in the group with the highest concentration of 2,2'4,4'-tetraBDE (p=0.09 for trend), there was no significant difference between cases and controls (p=0.14). The results for patients with malignant melanoma did not differ from controls.

Information on carcinogenic effects of PBDEs in animals is limited to results of chronic bioassays of decaBDE mixtures in rats and mice (Kociba et al. 1975; Norris et al. 1975b; NTP 1986). As summarized below, these studies provide limited evidence for the carcinogenicity of decaBDE in animals. No carcinogenicity studies of octaBDE or pentaBDE were located in the available literature.

The National Toxicology Program evaluated the carcinogenicity of commercial grade decaBDE (94-97% pure, no detected brominated dioxins or furans) in Sprague-Dawley rats (50/sex/dose) and B6C3F1 mice (50/sex/dose) that were exposed in the diet for 103 weeks and observed for an additional 0–1 weeks (NTP 1986). Comprehensive gross and histological examinations were performed on all animals in all dose groups including those that were moribund or died during the study. Reported estimated dose levels in the rats were 1,120 and 2,240 mg/kg/day in males and 1,200 and 2,550 mg/kg/day in females. Incidences of liver neoplastic nodules in low- and high-dose male rats (7/50 and 15/49, respectively) and high-dose female rats (9/50) were significantly greater than in controls (1/50 in both males and females) (p#0.03, Fisher Exact test) and showed positive dose-related trends ($p<0.001$, Cochran-Armitage trend test). Incidences of hepatocellular carcinoma alone (1/50, control males; 1/50, low-dose males; 1/49, high-dose males; 0/50, control females; 2/49, low-dose females; and 0/50, high-dose females) were not significantly increased in the treated rat groups compared to controls. The increased incidences of neoplastic nodules were considered as some evidence of compound carcinogenicity in both sexes. A dose-related trend for mononuclear cell leukemia was observed in treated male rats but was not considered to be biologically significant because of a high incidence in control animals.

Reported estimated doses in the mice were 3,200 and 6,650 mg/kg/day in males and 3,760 and 7,780 mg/kg/day in females (NTP 1986). Hepatocellular adenoma or carcinoma (combined) occurred at significantly increased incidences in low-dose male mice (22/50, $p=0.002$) and high-dose male mice (18/50, $p=0.019$) in comparison to controls (8/50) and showed a positive dose-related trend ($p=0.021$). Incidences of hepatocellular carcinoma alone were not significantly increased in either the low- or high-dose male mice. Slightly elevated incidences of thyroid gland follicular cell adenoma or carcinoma (combined) were additionally observed in exposed male mice but the increases were not statistically significant (control, 0/50; low dose, 4/50; high dose, 3/50). Incidences of follicular cell hyperplasia were significantly increased in male mice as summarized in the subsection on Endocrine Effects in Section 3.2.2.2. No significantly increased incidences of neoplastic lesions were observed in the female mice. NTP (1986) concluded that the significant increase in liver tumors and equivocal increase in thyroid tumors represented equivocal evidence of carcinogenicity in male mice. The evidence of carcinogenicity in the male mice was considered limited by an early loss of control animals. Losses of

control male mice were significant during the first year of the study but were subsequently comparable to the dosed mice; the early losses were presumed to be due to fighting among animals in both control and treatment groups.

The carcinogenicity of decaBDE was also evaluated in Sprague-Dawley rats (25/sex/dose) that were exposed to dietary doses of 0, 0.01, 0.1, or 1.0 mg/kg/day for approximately 2 years (702 days for males, 735 days for females) (Kociba et al. 1975; Norris et al. 1975b). The commercial mixture was comprised of 77.4% decaBDE, 21.8% nonaBDE, and 0.8% octaBDE and therefore differs from typical decaBDE formulations containing $97% decaBDE. Comprehensive histological examinations showed no exposure-related neoplastic effects. The ability of this study to detect carcinogenic changes is limited by the very low dose levels in comparison to those tested in the NTP (1986) bioassay.

The Cancer Effect Levels (CELs) for decaBDE in the NTP (1986) study are recorded in Table 3-3 and plotted in Figure 3-2.

3.2.3 Dermal Exposure

A few studies have examined groups of chemical workers involved in the manufacture and distribution of PBBs and/or PBDEs (Bahn et al. 1980; Brown et al. 1981; Chanda et al. 1982; Landrigan et al. 1979; Rosenman et al. 1979; Stross et al. 1981). Although the route of exposure (inhalation relative to dermal) of these workers has not been well defined, they appear to have had a high potential for dermal exposure (Anderson et al. 1978d). Results from these studies are discussed in this section, as well as in Section 3.2.1.

3.2.3.1 Death

Polybrominated Biphenyls. No reports of death in humans after dermal exposure to PBBs were located in the available literature.

No deaths were observed over a 14-day period among a group of four rabbits exposed to up to 10,000 mg/kg of body weight of a commercial octabromobiphenyl mixture by application to abraded and occluded dorsal trunk skin (Waritz et al. 1977). The bromobiphenyl was applied as a 35% (w/v) paste in corn oil. The same group of investigators reported that four of four rabbits died over a 14-day period after application of 5,000 mg/kg of a commercial hexabromobiphenyl mixture in the same vehicle as the octabromobiphenyl mixture. A dose of 10,000 mg/kg applied for 24 hours killed two of four rabbits. The cause of death was not reported. A commercial mixture of decabromobiphenyl in corn oil was not lethal in rats that were observed for 14 days following application of a single dose as high as 5,000 mg/kg to covered intact skin (Millischer et al. 1980). The octabromobiphenyl LOAEL of 5,000 mg/kg is reported in Table 3-2. It is unclear whether the different lethality rates observed among the hexa-, octa-, and decabromobiphenyl mixtures reflect differences in lethal potency or in absorption rates or both.

Table 3-3. Levels of Significant Exposure to PBBs and PBDEs - Dermal

Species (Strain)	Exposure/ Duration/ Frequency	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
ACUTE EXPOSURE						
Death						
Rabbit (NZW)	24 hr			5000	(4/4 died)	Waritz et al. 1977 (HBB)
Systemic						
Rabbit (Albino)	once	Derm	658			Millischer et al. 1980 (DBB)
Rabbit (NZW)	5 d 1x/d	Derm		0.19 (hyperkeratosis)		Needham et al. 1982 (FF-1)
Rabbit (NS)	2 wk 5 d/wk 1x/d	Hepatic		1 (increased liver weight)		Waritz et al. 1977 (OBB)
		Derm	1			
		Bd Wt	1			
Rabbit (NZW)	24 hr	Hepatic	100 M	1000 (increased liver weight; necrotic foci)		Waritz et al. 1977 (HBB)
		Bd Wt	1000 M	5000 (11% weight loss)		

Table 3-3. Levels of Significant Exposure to PBBs and PBDEs - Dermal (continued)

Species (Strain)	Exposure/ duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
				Less serious (mg/kg/day)	Serious (mg/kg/day)	
INTERMEDIATE EXPOSURE						
Systemic						
Gn pig (Albino Hartley)	3 wk 3x/wk	Derm	62 M			Waritz et al. 1977 (OBB)

Bd Wt = body weight; d = days; Derm = dermal; hr = hour(s); M = male; wk = week(s); x = time(s)

Polybrominated Diphenyl Ethers. No reports of death in humans after dermal exposure to PBDEs were located in the available literature.

No deaths occurred in rabbits that were observed for 14 days following a single #2,000 mg/kg dermal dose of decaBDE, octaBDE or pentaBDE (IRDC 1974, 1975a, 1975b). The PBDEs were applied to clipped intact skin, covered with an occlusive barrier, and washed from the treatment site 24 hours later.

3.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, or renal effects in humans or animals after dermal exposure to PBBs.

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, or renal effects in humans or animals after dermal exposure to PBDEs.

Systemic effects that have been observed in humans and animals following dermal exposure to PBBs and PBDEs are described below. The highest NOAEL and all LOAEL values from each reliable study for systemic end points in each species and duration category are recorded in Table 3-3.

Hepatic Effects.

Polybrominated Biphenyls. No studies were located regarding hepatic effects in humans after dermal exposure to PBBs.

No significant changes in relative or absolute liver weight or gross pathological effects were reported in groups of four rabbits after application of a single dose of up to 10,000 mg/kg of octabromobiphenyl mixture in corn oil to abraded and occluded dorsal skin over a 24-hour period (Waritz et al. 1977). It was unclear if histopathological examinations were performed. Using the same protocol in rabbits, these investigators reported a significant increase ($p<0.01$) in relative and absolute liver weight, distinct lobular markings, and necrotic foci with doses $1,000 mg/kg of a commercial hexachlorobiphenyl mixture. A dose of 100 mg/kg was without effect. A significant increase ($p<0.01$) in relative liver weight was reported in rabbits after application of 1 mg/kg/day of a commercial mixture of octabromobiphenyl in corn oil to the intact and occluded shaved dorsal skin on 5 days/week for 2 weeks (Waritz et al. 1977). Histopathological examinations were not performed. A relatively low dose (0.0013 mg/kg) of FireMaster

BP-6 dissolved in benzene/decame (1:9) applied once a day for 5 days to the ear of three rabbits caused no histopathological effects in the liver (Hass et al. 1978).

No studies were located regarding hepatic effects following intermediate or chronic dermal exposure to PBBs.

Endocrine Effects.

Polybrominated Biphenyls. Hypothyroidism was diagnosed in 4 of 35 men who were occupationally exposed to unspecified PBBs and/or decaBDE (Bahn et al. 1980). The cohort consisted of workers (mean age 35.9 years) who had been employed at a production plant for at least 6 weeks during a 52-month period during which PBBs and decaBDE were the only chemicals manufactured and who had volunteered for a comprehensive medical evaluation performed 3 months after the end of the 52-month period. There was no further description of exposure, and it was assumed to have occurred by both inhalation and dermal routes. As detailed in Section 3.2.1.2, the results of this study suggest that occupational exposure to PBBs, decaBDE, and/or bromine affected the thyroid, but the mixed chemical exposure and a lack of data on serum or tissue levels of the chemicals preclude attributing effects solely to any particular congener or mixture of congeners.

No studies were located regarding endocrine effects in animals after dermal exposure to PBBs.

Polybrominated Diphenyl Ethers. There is suggestive evidence of hypothyroidism in a small group of workers who were occupationally exposed to decaBDE as well as PBBs (Bahn et al. 1980) as summarized above and detailed in Section 3.2.1.2.

No studies were located regarding endocrine effects in animals after dermal exposure to PBDEs.

Dermal Effects.

Polybrominated Biphenyls. As discussed in Section 3.2.1.2, results from a medical history survey study of workers in a PBB manufacturing plant and a nonexposed group of Wisconsin farm residents indicated an association between occupational exposure to PBBs and the occurrence of acne (Chanda et al. 1982). The survey covered a period of 3 years of potential exposure, but exposure levels were not reported. No adverse dermal effects were observed on the arms or legs of subjects after a 6-day application of polymer

fibers containing commercial octabromobiphenyl mixture under an occlusive covering; no additional information was reported (Waritz et al. 1977).

Several studies examined the acute dermal effects of commercial PBB mixtures in rabbits. Application of 0.19 mg/kg FireMaster FF-1 for 5 days, in toluene vehicle, to the inner surface of the left ear of two rabbits (right ear served as control) induced moderate hyperkeratosis, which included marked dilation of the hair follicles, with moderate proliferation of the epithelium and partial atrophy of the sebaceous glands (Needham et al. 1982). There was also evidence of excess keratin and debris in the subjacent hair follicles. Application of either a dry or water-moistened formulation of octabromobiphenyl mixture (amount not reported) for 24 hours did not adversely affect intact skin in rabbits, but slight erythema and edema were observed in abraded skin (Norris et al. 1975a). Repeated applications over a 2-week period of the dry octabromobiphenyl mixture formulation (amount not reported) to occluded intact or abraded skin caused no skin response, but the water-moistened formulation caused slight and transient erythema (Norris et al. 1975a). None of these studies reported the number of animals used. Rough skin with mild erythema was observed in occluded intact shaved dorsal skin of a group of rabbits after repeated applications of a dose of 1 mg/kg/day octabromobiphenyl mixture in corn oil over a 2-week period (Waritz et al. 1977). Application of a commercial decabromobiphenyl mixture in olive oil to covered intact or abraded skin for 4 hours, in an amount equivalent to 658 mg/kg, caused very slight erythema with or without edema in rabbits (Millischer et al. 1980).

Limited information is available regarding intermediate-duration dermal effects of PBBs in animals. A 10% chloroform solution of an unspecified commercial formulation of octabromobiphenyl did not induce bromacne when applied to the ear of rabbits for 30 days (Norris et al. 1975a). Only slight erythema and exfoliation was observed. Doses of 62 mg/kg of octabromobiphenyl mixture were not sensitizing when applied to the intact or abraded skin of guinea pigs over a 3-week period (Waritz et al. 1977).

No studies were located regarding dermal effects in animals after chronic application of PBBs.

Polybrominated Diphenyl Ethers. A human sensitization study was conducted in which 77.4% decaBDE (containing 21.8% nonaBDE and 0.8% octaBDE) as a 5% suspension in petrolatum was applied via patch, 3 times a week for 3 weeks, to 50 subjects (Norris et al. 1975a). No skin sensitization responses occurred during the sensitizing period or on challenge 2 weeks following the last application. No additional information was reported regarding the design and results of this study.

There was no evidence of primary irritation in intact skin of rabbits that were dermally exposed to decaBDE (500 mg as dry solid was applied to clipped skin and occluded for 24 hours) (IRCD 1974). Similar application of 77.4% decaBDE (containing 21.8% nonaBDE and 0.8% octaBDE) (dry solid, amount not reported), octaBDE (500 mg as dry solid), or pentaBDE (0.5 mL as a viscous liquid) was also non-irritating to intact rabbit skin (IRCD 1975a, 1975b; Norris et al. 1975a). Similar application of solid 77.4% decaBDE (21.8% nonaBDE and 0.8% octaBDE) or octaBDE to abraded skin caused a slight erythematous and edematous response in rabbits (Norris et al. 1975a). OctaBDE moistened with water caused no response on intact skin and a moderate erythematous and slight edematous response on abraded skin after 24 hours of occluded contact in rabbits (Norris et al. 1975a).

Repeated applications of solid 77.4% decaBDE (21.8% nonaBDE and 0.8% octaBDE) or octaBDE to intact skin (5 days/week for 2 weeks) or abraded skin (3 days) was non-irritating to rabbits (Norris et al. 1975a). Similar repeated applications of octaBDE moistened with water caused a slight erythematous response on intact skin and a moderate erythematous and slight edematous response on abraded skin. All skin sites returned to normal appearance following cessation of exposure.

OctaBDE and pentaBDE were non-sensitizing in maximization tests in guinea pigs (Microbiological Associates Inc. 1996). The induction doses consisted of three pairs of interscapular region intradermal injections of (1) a 50:50 solution of Freund's adjuvant and corn oil, (2) 2.5% octaBDE or 5% pentaBDE solutions in corn oil, and (3) 2.5% octaBDE or 5% pentaBDE in the 50:50 corn oil/Freund's adjuvant solution. Control groups received the same regimen without PBDEs. After 7 days, the PBDE-treated animals received topical applications of neat octaBDE or pentaBDE on the previously treated interscapular sites. Two weeks later, the animals were challenged with topical doses of neat octaBDE or pentaBDE on the left flank. Subsequent examination of the test sites at 24, 48, 72, 96, or 120 hours after the challenge dose showed no erythema or edema responses in any of the animals, indicating that the PBDEs did not cause delayed contact hypersensitivity.

A 10% chloroform solution of 77.4% decaBDE (containing 21.8% nonaBDE and 0.8% octaBDE) or octaBDE did not induce bromacne when applied to the ear of rabbits for 30 days (Norris et al. 1975b). A slight erythematous response and slight exfoliation were the only observed effects. No additional information was reported on the design and results of this acnegenesis study.

Ocular Effects.

Polybrominated Biphenyls. No studies were located regarding ocular effects in animals after dermal exposure to PBBs.

Transient irritation of the conjunctival membranes was observed after a single instillation of an unreported amount of dry solid octabromobiphenyl mixture to the eye in rabbits, but the cornea, iris, and lens were unaffected (Norris et al. 1975a). Commercial grade decabromobiphenyl did not cause eye irritation in rabbits when 0.05 mg in olive oil was instilled for 30 seconds followed by rinsing, but application of an unspecified amount of dry powder without rinsing was slightly irritating (Millischer et al. 1980). Mild conjunctival redness and swelling and a copious discharge was reported after application of 100 mg of an unspecified commercial PBB powder mixture (either hexa- or octabromobiphenyl) for 20 seconds into the conjunctival sac of two rabbits (Waritz et al. 1977). These effects disappeared within 4 hours in both washed (with tap water) and unwashed eyes. The iris and cornea were unaffected.

Polybrominated Diphenyl Ethers. No studies were located regarding ocular effects in animals after dermal exposure to PBDEs.

Ocular effects were investigated in rats that had 100 mg decaBDE (solid), 100 mg octaBDE (solid), or 0.1 mL pentaBDE (viscous liquid) instilled into the conjunctival sac (ICRD 1974, 1975a, 1975b). The eyes were examined for irritation after 24, 48, and 72 hours and 7 days and corneal injury after 72 hours. There were no exposure-related effects with decaBDE or octaBDE, although pentaBDE caused slight evidence of corneal damage in one of six rats (IRDC 1975b).

Body Weight Effects.

Polybrominated Biphenyls. No studies were located regarding body weight effects in humans after dermal exposure to PBBs.

No treatment-related effects on body weight were reported in rabbits given a dose of 1 mg/kg/day of a commercial mixture of octabromobiphenyl in corn oil via application to the intact shaved dorsal skin for 2 weeks (Waritz et al. 1977). No significant effect on final body weight was reported in groups of four rabbits after application of a single dose of up to 10,000 mg/kg of octabromobiphenyl mixture in corn oil to the abraded and occluded dorsal skin over a 24-hour period (Waritz et al. 1977). The observation

period was 14 days. In a similar study with a commercial mixture of hexabromobiphenyl, rabbits treated with 1,000 mg/kg showed no weight gain over 14 days. Doses of 5,000 and 10,000 mg/kg induced an 11% and 20% weight loss, respectively, whereas, a dose of 100 mg/kg was without effect (Waritz et al. 1977).

Polybrominated Diphenyl Ethers. No studies were located regarding body weight effects in humans after dermal exposure to PBDEs.

There were no adverse effects on body weight in rabbits that were observed for 14 days following a single #2,000 mg/kg dermal dose of decaBDE, octaBDE, or pentaBDE (IRDC 1974, 1975a, 1975b). The PBDEs were applied to clipped intact skin, covered with an occlusive barrier, and washed from the treatment site after 24 hours.

3.2.3.3 Immunological and Lymphoreticular Effects

Polybrominated Biphenyls. Several immunological parameters were evaluated in a group of 28 workers from the Michigan Chemical Company who were involved in the manufacturing and distribution of PBBs including FireMaster FF-1 (Stross et al. 1981). It is assumed that the main route of exposure was dermal, but inhalation and/or oral exposure cannot be ruled out. The subjects had worked directly with PBBs during the previous 5 years. Immunological studies included determination of immunoglobulin levels, skin testing, and lymphocyte transformation studies. No abnormalities in lymphocyte number or function could be determined when compared to an unexposed group. One of three blastogenic responses (PWM) was significantly reduced ($p<0.01$) relative to controls, but was within the normal range for the laboratory. No specific information was provided regarding the skin testing and immunoglobulin levels.

No studies were located regarding immunological effects in animals after dermal exposure to PBBs.

Polybrominated Diphenyl Ethers. No studies were located regarding immunological effects in humans or animals after dermal exposure to PBDEs.

3.2.3.4 Neurological Effects

Polybrominated Biphenyls. Twenty-five workers at a PBB manufacturing plant displayed mean scores on tests of memory and learning that were typical for people of their age, educational, occupational, and cultural backgrounds, even though they displayed an elevated mean PBB concentration in adipose tissue (9.33 ppm compared with 3.94 ppm for farm residents) (Brown et al. 1981). Workers with the highest concentrations of PBB in adipose tissue showed no evidence of memory dysfunction in these tests. Because 15/25 "directly handled PBBs or performed maintenance work in the area where PBBs were manufactured," it is likely that at least part of the exposure was by the dermal route.

No studies were located regarding neurological effects in animals after dermal exposure to PBBs.

Polybrominated Diphenyl Ethers. No studies were located regarding neurological effects in humans or animals after dermal exposure to PBDEs.

3.2.3.5 Reproductive Effects

Polybrominated Biphenyls. Eleven workers in a PBB manufacturing company in Michigan displayed no differences in the distribution of sperm counts, motility, or morphology compared with a control group of 52 unexposed men (Rosenman et al. 1979). PBBs were detected in the serum of all exposed subjects and in only one unexposed subject, but mean or individual serum PBB values were not reported.

No studies were located regarding reproductive effects in animals after dermal exposure to PBBs.

Polybrominated Diphenyl Ethers. No studies were located regarding reproductive effects in humans or animals after dermal exposure to PBDEs.

3.2.3.6 Developmental Effects

Polybrominated Biphenyls. No studies were located regarding developmental effects in humans or animals after dermal exposure to PBBs.

Polybrominated Diphenyl Ethers. No studies were located regarding developmental effects in humans or animals after dermal exposure to PBDEs.

3.2.3.7 Cancer

Polybrominated Biphenyls. No studies were located regarding cancer in humans after dermal exposure to PBBs.

An unspecified PBB mixture (purity not reported) was not tumorigenic when applied to the shaved dorsal skin of female CD-1 mice at a dose of 3.3 mg/kg twice weekly for 30 weeks; no tissues other than skin were examined (Berry et al. 1978, 1979). This same treatment did not promote the development of skin tumors in mice pretreated with a single application of a tumor initiator, dimethylbenzanthracene (DMBA), 1 week prior to PBB exposure (Berry et al. 1978, 1979). The results of these studies must be interpreted with caution, since a dose-response study was not done (i.e., only one dose level was tested, and the doses may have been too low). Toxic doses of FireMaster FF-1 promoted development of skin tumors in female HRS/J hairless mice (Poland et al. 1982). A single dermal application of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) initiator, followed by twice weekly applications of FireMaster FF-1 at 66.7 mg/kg for 5 weeks and then 33.3 mg/kg for 15 weeks, resulted in a 60% (9/15) incidence of papillomas compared to 0% (0/23) in MNNG-only controls. Toxic effects included mortality, which caused the dose reduction after 5 weeks, and severe hepatomegaly and hepatic porphyria.

Polybrominated Diphenyl Ethers. No studies were located regarding cancer in humans or animals after dermal exposure to PBDEs.

3.3 GENOTOXICITY

Polybrominated Biphenyls. No studies were located regarding genotoxic effects in humans following inhalation, oral, or dermal exposure to PBBs.

In vivo genotoxicity studies of PBBs in animals are summarized in Table 3-4. Administration of single oral doses between 50 and 1,000 mg of FireMaster FF-1/kg (purity not reported) by gavage in corn oil to male and female B6C3F1 mice and male Fischer-344 rats did not induce unscheduled deoxyribonucleic acid (DNA) synthesis in hepatocytes (Mirsalis et al. 1985, 1989). However, doses $200 mg/kg significantly increased hepatic cell proliferation in mice, but not in rats. The increase in cell proliferation without a change in unscheduled DNA synthesis suggests that PBBs acted as a promoter rather than directly causing DNA damage (initiator). A commercial mixture of decabromobiphenyl did not induce gene mutation in *Salmonella typhimurium* bacteria that were intraperitoneally injected into male CFLP mice in a host-mediated assay (Millischer et al. 1980). This decabromobiphenyl mixture also did not induce micronuclei in bone marrow erythrocytes of mice (Millischer et al. 1980). The mice in the host-mediated assay and micronucleus test were orally treated (method not specified) with total doses of 5,000, 10,000, or 20,000 mg/kg, administered in two equal doses 24 hours apart.

In vitro studies indicate that PBBs are not directly genotoxic. As summarized in Table 3-5, PBBs did not exhibit mutagenic activity when tested in the prokaryotic organisms *S. typhimurium* (Haworth et al. 1983; Millischer et al. 1980; NTP 1983) and *E. coli* (Rossman et al. 1991) with or without activation systems in the limited number of studies available. *In vitro* testing in eukaryotic cells resulted in negative genotoxic responses in hamster cells (Galloway et al. 1987; Kavanagh et al. 1985; Williams et al. 1984), rat liver cells (Kavanagh et al. 1985; Williams et al. 1984), mouse liver and lymphoma cells (Myhr and Caspary 1991; Williams et al. 1984), and human fibroblasts (Williams et al. 1984).

An *in vitro* study with a ^{14}C-PBB mixture containing 12 major components found only traces of radioactivity bound to rat liver microsomal macromolecules (Dannan et al. 1978a). Binding, however, was dependent upon the type of microsomes used to activate the PBB mixture. Microsomes isolated from animals pretreated with methylcholanthrene (MC) bound twice the amount of radioactivity compared with controls, whereas activation with phenobarbital (PB) or PBB bound 5 times more radioactivity than control microsomes. Also, the authors showed that no radioactivity was covalently bound to DNA following incubation with ^{14}C-PBB. The type of microsomes used or the presence or

Table 3-4. Genotoxicity of PBBs and PBDEs *In Vivo*

Species (test system)	End point	Results	Reference
PBBs			
Mammalian cells:			
Rat hepatocytes	Unscheduled DNA synthesis	–	Mirsalis et al. 1989 (FF-1)
Mouse hepatocytes	Unscheduled DNA synthesis	–	Mirsalis et al. 1985, 1989 (FF-1)
Host-mediated assays:			
Salmonella typhimurium (mouse hosted-mediated)	Gene mutation	–	Millischer et al. 1980 (DBB)
Micronucleus test:			
Mouse bone marrow erythrocytes	Chromosome aberration (micronuclei)	–	Millischer et al. 1980 (DBB)
PBDEs			
Cytogenicity:			
Rat bone marrow cells (one-generation reproduction study)	Chromosome aberration	–	Norris et al. 1973, 1975 (77.4% decaBDE, 21.8% nonaBDE)

– = negative result; DBB = decabromobiphenyl commericial mixture; decaBDE = decabromodiphenyl ether; DNA = deoxyribonucleic acid; FF-1 = FireMaster FF-1; nonaBDE = nonabromodiphenyl ether; PBBs = polybrominated biphenyls; PBDEs = polybrominated diphenyl ethers

Table 3-5. Genotoxicity of PBBs and PBDEs *In Vitro*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
PBBs				
Prokaryotic organisms:				
Salmonella typhimurium (plate incorporation)	Gene mutation	–	–	NTP 1983 (FF-1)
S. typhimurium (plate incorporation)	Gene mutation	–	–	Haworth et al. 1983 (HBB)
S. typhimurium (plate incorporation)	Gene mutation	–	–	Millischer et al. 1980 (DBB)
Escherichia coli (culture)	Gene mutation	–	–	Rossman et al. 1991 (PBB)
Eukaryotic organisms				
Mammalian cells:				
Chinese hamster CHO cells (cell culture)	Chromosomal aberration	–	–	Galloway et al. 1987 (HBB)
Chinese hamster CHO cells (cell culture)	Sister chromatid exchange			Galloway et al. 1987 (HBB)
Chinese hamster V79 cells (cell culture)	Gene mutation	–	–	Kavanagh et al. 1985 (BP-6)
Chinese hamster V79 cells (cell culture)	Gene mutation	No data	–	Kavanagh et al. 1985 (2,4,5-HBB)
Chinese hamster V79 cells (cell culture)	Gene mutation	No data	–	Kavanagh et al. 1985 (3,4,5-HBB)
Chinese hamster V79 cells (cell culture)	Gene mutation	–	–	Kavanagh et al. 1985 (3,4-TBB)
Rat liver cells WB (cell culture)	Gene mutation	No data	–	Kavanagh et al. 1985 (2,4,5-HBB)
Rat liver cells WB (cell culture)	Gene mutation	No data	–	Kavanagh et al. 1985 (3,4,5-HBB)
Mouse lymphoma cells L5178Y (cell culture)	Gene mutation	–	–	Myhr and Caspary 1991 (FF-1)
Rat liver cells (cell culture)	DNA repair	No data	–	Williams et al. 1984 (FF-1)
Mouse liver cells (cell culture)	DNA repair	No data	–	Williams et al. 1984 (FF-1)
Hamster liver cells (cell culture)	DNA repair	No data	–	Williams et al. 1984 (FF-1)
Rat liver cells (cell culture)	Gene mutation	No data	–	Williams et al. 1984 (FF-1)
Human fibroblast D-550 (cell culture)	Gene mutation	–	No data	Williams et al. 1984 (FF-1)

Table 3-5. Genotoxicity of PBBs and PBDEs *In Vitro (continued)*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
PBDEs				
Prokaryotic organisms: *Salmonella typhimurium* (plate incorporation)	Gene mutation	–	–	NTP 1986 (decaBDE)
Mammalian cells: Mouse lymphoma L5178Y cells (cell culture)	Gene mutation	–	–	NTP 1986 (decaBDE)
Chinese hamster Sp5/V79cells (cell culture)	Gene recombination	no data	-	Helleday et al. 1999 (2,2',4,4'-tetraBDE)
Chinese hamster SPD8/V79cells (cell culture)	Gene recombination	no data	%	Helleday et al. 1999 (2,2',4,4'-tetraBDE)
Chinese hamster Sp5/V79 cells (cell culture)	Gene recombination	no data	%	Helleday et al. 1999 (3,4-diBDE)
Chinese hamster SPD8/V79 cells (cell culture)	Gene recombination	no data	%	Helleday et al. 1999 (3,4-diBDE)
Chinese hamster Sp5/V79 cells (cell culture)	Gene recombination	no data	%	Helleday et al. 1999 (2-monoBDE)
Chinese hamster SPD8/V79 cells (cell culture)	Gene recombination	no data	%	Helleday et al. 1999 (2-monoBDE)
Chinese hamster ovary cells (cell culture)	Sister chromatid exchange	–	–	NTP 1986 (decaBDE)
Chinese hamster ovary cells (cell culture)	Chromosomal aberrations	–	–	NTP 1986 (decaBDE)

– = negative result; 2,2',4,4'-tetraBDE = 2,2',4,4'-tetrabromodiphenyl ether; 2,4,5-HBB = 2,2',4,4',5,5'-hexabromobiphenyl; 2-monoBDE = 2-bromodiphenyl ether; 3,4,5-HBB = 3,3',4,4',5,5'-hexabromobiphenyl; 3,4-diBDE = 3,4-dibromodiphenyl ether; 3,4-TBB = 3,3',4,4'-tetrabromobiphenyl; BDE = brominated diphenyl ethers; BP-6 = FireMaster BP-6; DBB = decabromobiphenyl commercial mixture; decaBDE = decabromobiphenyl ether; DNA = deoxyribonucleic acid; FF-1 = FireMaster FF-1; HBB = hexabromobiphenyl (unspecified); PBB = unspecified mixture; PBBs = polybrominated biphenyls; PBDEs = polybrominatedd diphenyl ethers

absence of nicotinamide adenine dinucleotide phosphate (reduced form) (NADPH) in the incubation mixture made no difference.

Although it appears that PBBs are not mutagenic, due to their enzyme induction properties, they may potentiate the genotoxic activity of other compounds by activation to reactive intermediates.

Polybrominated Diphenyl Ethers. No studies were located regarding genotoxic effects in humans following inhalation, oral, or dermal exposure to PBDEs.

A limited amount of information has been published on the genotoxicity of PBDEs in animals *in vivo* or in prokaryotic and eukaryotic cells *in vitro* as summarized in Tables 3-4 and 3-5, respectively. Cytogenetic examination of bone marrow cells showed no increase in aberrations in maternal and neonatal rats following maternal oral exposure to #100 mg/kg/day of a 77.4% decaBDE mixture (containing 21.8% nonaBDE and 0.8% octaBDE) for 90 days prior to mating and during mating, gestation, and lactation (Norris et al. 1973, 1975a). *In vitro* assays found that decaBDE did not induce gene mutations in bacterial cells (*S. typhimurium TA98, TA100, TA1535,* or *TA1537*) or mammalian cells (mouse lymphoma L5178Y cells), and did not induce sister chromatid exchange or chromosomal aberrations in Chinese hamster ovary cells (NTP 1986). *In vitro* exposure to the congeners 2,2',4,4'-tetraBDE, 3,4-diBDE, and 2-monoBDE caused increased recombinogenic activity at the HGPRT locus in Chinese hamster SPD8 and Sp5 V79 cells (Helleday et al. 1999).

3.4 TOXICOKINETICS

Data regarding the toxicokinetics of PBBs in humans are limited to information derived from cases of accidental ingestion of food contaminated with PBBs and cases of occupational exposure by the inhalation and dermal routes. These data provide qualitative evidence that PBBs are absorbed in humans by the inhalation, oral, and dermal routes. Limited quantitative data in animals indicate that some PBB congeners are well absorbed after oral exposure. Dermal absorption data for animals are insufficient for estimating absorption rates, and no inhalation absorption data were located. In blood, 80% of PBBs, are bound to protein and 20% are associated with lipids. The distribution pattern of PBBs did not differ significantly between humans and animals and among animal species. Due to their lipophilic nature, PBBs, especially the highly brominated congeners, tend to accumulate in lipid-rich tissues. Greater relative amounts of PBBs are usually found in the liver, adipose, skin, and breast milk. Certain components of PBB mixtures are metabolized by the microsomal monooxygenase system catalyzed by cytochrome P-450 of the type induced by phenobarbital. The rate of metabolism of some PBB congeners

depends on the bromine substitution pattern. PBB congeners of low bromine content are transformed into hydroxylated derivatives that are predominately eliminated in the urine. Highly brominated congeners are either retained or excreted unchanged in the feces. The exact mechanism of PBB toxicity is not known. It has been suggested, however, that the mechanism for some congeners is related to the enhancement of gene expression triggered by initial binding to the same cytosolic receptor (Ah) involved in PCB and polychlorinated dioxin toxicity.

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

Polybrominated Biphenyls. No studies were located regarding absorption of PBBs in humans after inhalation exposure. However, absorption of PBBs by inhalation (and by dermal contact) in humans can be inferred by the relatively high levels of PBB residues detected in adipose tissue and serum of workers involved in PBB manufacturing (Brown et al. 1981; Landrigan et al. 1979; Stross et al. 1981).

No studies were located regarding quantitative absorption of PBBs in animals after inhalation exposure to PBBs. However, increased bromine concentrations were found in the liver and adipose tissue of rats exposed continuously to a commercial mixture of octabromobiphenyl for 15 weeks, suggesting that absorption had occurred (Waritz et al. 1977).

Polybrominated Diphenyl Ethers. No studies were located regarding absorption of PBDEs in humans or animals after inhalation exposure.

3.4.1.2 Oral Exposure

Polybrominated Biphenyls. Quantitative oral absorption data in humans were not located, but reports of increased levels of PBB residues in tissues and serum of individuals accidentally exposed to contaminated food indicate that gastrointestinal absorption of PBBs had occurred (Eyster et al. 1983; Humphrey and Hayner 1975; Landrigan et al. 1979; Miceli et al. 1985; Wolff et al. 1982).

Absorption of PBBs from the gastrointestinal tract in animals can be inferred from the numerous reports of adverse effects (Section 3.2.2) and increased residue levels in tissues following oral administration of these compounds (Section 3.4.2.2); however, few quantitative data exist. By comparing the amount of

radioactivity in the feces of rats administered a single oral dose of 1 mg ^{14}C-2,2',4,4',5,5'-hexabromobiphenyl/kg with that monitored after a single intravenous injection of the compound, it was estimated that . 93% of the oral dose was absorbed over a 24-hour period (Matthews et al. 1977). Data obtained from similar experiments later confirmed these results (Tuey and Matthews 1980). It was also demonstrated that absorption of the hexabromobiphenyl congener was independent of the dose, since $90% was absorbed over a dose range of 1–30 mg/kg (Matthews et al. 1977). In contrast with the high absorption rate for the hexabromobiphenyl congener, octabromobiphenyl (45.2% octa, 47% nona, 5.7% deca, 1.8% hepta) appeared to be less well absorbed by rats after administration of a single dose of 1 mg/kg (Norris et al. 1975a). Within the first 24 hours after dosing, 61.9% of the dose was found in the feces. This indicates that at least 38.1% of the dose was absorbed, but absorption may have been higher, since biliary excretion may have occurred.

Polybrominated Diphenyl Ethers. No information was located regarding absorption of PBDEs in humans following oral exposure. Limited information regarding absorption in animals has been inferred from data on elimination and excretion. Results from these studies suggest that decaBDE is poorly absorbed in rats. For example, 24 hours following administration of a single gavage dose of ^{14}C-decaBDE (commercial mixture), approximately 91% of decaBDE-derived radioactivity was found in the feces (Norris et al. 1975b). In the same time period, approximately 62% of a dose of octaBDE was detected in the feces, suggesting that octaBDE is more efficiently absorbed than decaBDE. Studies conducted with reagent-grade decaBDE provided similar results (El Dareer et al. 1987). The less brominated 2,2',4,4'-tetraBDE was much more efficiently absorbed by rats and mice since only 5 and 7% of a single dose was found in the feces 24 hours after dosing (Örn and Klasson-Wehler 1998).

3.4.1.3 Dermal Exposure

Polybrominated Biphenyls. No studies were located regarding absorption of PBBs in humans or animals after dermal exposure to PBBs. However, absorption of PBBs through the skin in humans can be inferred by the relatively high levels of PBB residues detected in the adipose tissue and serum of workers involved in the manufacturing of these chemicals (Brown et al. 1981; Landrigan et al. 1979; Stross et al. 1981). It is assumed that dermal route predominates, but inhalation and/or oral exposure cannot be ruled out.

Similarly, dermal absorption in rabbits can be inferred from reports of lethality and liver effects observed after application of a commercial mixture of hexabromobiphenyl to abraded and occluded dorsal skin (Waritz et al. 1977).

Polybrominated Diphenyl Ethers. No information was located regarding dermal absorption of PBDEs in humans. The only information regarding dermal absorption in animals is that from a study of absorption in an *in vitro* preparation (Hughes et al. 2001). In that study, ^{14}C-decaBDE dissolved in tetrahydrofuran was applied to dorsal skin (three dose levels) excised from adult hairless female mice and fractions of receptor fluid were collected over a 24-hour period. Transfer of radioactivity to the receptor fluid was minimal, only 0.07 to 0.34% of the applied radioactivity. Two to 20% of the radioactivity was found in the skin, and the lowest dose applied had the highest percentage of the dose in the skin. Washing the skin with solvent 24 hours after application removed 77–92% of the applied dose. In this study, decaDBE did not easily penetrate the skin, but inferences to dermal absorption in humans based on these limited results may not be appropriate.

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

Polybrominated Biphenyls. No studies were located regarding distribution of PBBs in humans after inhalation exposure.

Limited information was located regarding distribution of PBBs in animals after inhalation exposure. Increased bromide concentrations were observed in the liver and adipose tissue of rats exposed continuously to vapors of a commercial octabromobiphenyl mixture (33% octa, 60% nona, 6% deca, 1% hepta) (3.5 pg octabromobiphenyl/L air at equilibrium) for 15 weeks (Waritz et al. 1977). Relative to controls, the concentration of bromide in liver and fat was increased by 39 and 100%, respectively; bromide concentration in skeletal muscle was not affected by treatment. No further details were provided.

Polybrominated Diphenyl Ethers. No information was located regarding distribution of PBDEs in humans following inhalation exposure.

The distribution of bromine was examined in tissues of rats after inhalation exposure octaBDE (IRDC 1978). Groups of rats were exposed to 0, 1.2, 12, 120, or 1,200 mg/m^3 of dusts of octaBDE 8 hours/day for 14 days. At necropsy, sections of the lungs, adipose tissue, and liver were collected for bromine analysis using a neutron activation technique. The results showed concentrations of bromine in the lungs and adipose tissue significantly higher in all groups relative to controls; the amounts of bromine detected

were concentration-related. In the liver, the concentration of bromine was also elevated in all groups relative to controls except in the 1.2 mg/m^3 exposure group; the increases in the liver were not as marked as in the lungs or in adipose tissue.

3.4.2.2 Oral Exposure

Polybrominated Biphenyls. Numerous reports have been published regarding levels of PBBs in serum, adipose tissue, breast milk, placenta, and cord serum of humans exposed to PBBs via the diet (Anderson et al. 1978d; Eyster et al. 1983; Landrigan et al. 1979; Stross et al. 1979, 1981; Wolff et al. 1979a, 1982). By using paired sampling, several significant correlations were determined (Eyster et al. 1983). For example, in parturient women from Michigan, statistically significant correlations were found between PBB levels in maternal serum and placenta, cord serum, breast milk, and adipose; and between PBB levels in adipose tissue and breast milk. In addition, there was a significant correlation between PBB levels in serum and feces and between serum and biliary fluid samples in farmers and chemical workers in Michigan. In groups of pregnant, nonpregnant, and male chemical workers the serum to adipose tissue PBB concentration ratios ranged from 1:140 to 1:160, but in male farmers, this ratio was 1:325–329 (Eyster et al. 1983). The latter value is consistent with other reports regarding Michigan populations (Landrigan et al. 1979; Wolff et al. 1982). It is unclear why the partitioning ratios between male chemical workers and farmers should differ. The investigators noted that the group of farmers was much larger and might represent a better sample, as well as the possibilities that the farmers may have been more physically active or, for a variety of reasons, may have had lower total serum lipids (the amount of serum lipid might have affected the serum concentration of PBBs). PBB levels in body tissues and fluids are further discussed in Section 6.4.

Analysis of postmortem tissue samples from 15 subjects in the Grand Rapids, Michigan area indicated that renal fat had the highest single PBB concentration (1.65 µg/g wet weight) and the highest mean concentration (0.475 µg/g) (Miceli et al. 1985). In regards to adipose, PBB concentrations in different tissues, could be divided into three range groups: high (ratios of 0.45–0.56, adrenal, atheromatous aorta, and thymus), medium (ratios of 0.1–0.28, pancreas, liver, and left ventricle), and low (ratios of 0.02–0.09, kidney, lung, brain, skeletal muscle, thyroid, and nonatheromatous aorta).

As with the structurally related PCBs (ATSDR 2000), PBBs are rapidly (minutes to hours) cleared from the blood and initially accumulate mainly in the liver, lungs, and muscle (Domino et al. 1982; Matthews et al. 1977). Due to their high affinity for lipid-rich tissues, PBBs are subsequently redistributed to

adipose and skin for storage or metabolism in the liver, and a dynamic equilibrium of PBB concentrations is established among all tissues for each PBB homolog (Tuey and Matthews 1980).

In rats treated by gavage with one or four daily doses of ^{14}C-2,2',4,4',5,5'-hexabromobiphenyl, initial concentrations of radioactivity were highest in muscle, liver, and adipose tissue, but later redistribution to adipose tissue (4–7 days after the last dosing) resulted in lower concentrations in liver and muscle (Matthews et al. 1977). In rats dosed daily with ^{14}C-2,2',4,4',5,5'-hexabromobiphenyl over a 30-day period, tissue concentrations on day 31 were (in increasing order): blood, muscle, liver, skin, and adipose and were in general agreement with those predicted by a physiological compartment model (Tuey and Matthews 1980). When the model was scaled to nonlactating humans as discussed in Section 3.4.5 (Physiologically Based Pharmacokinetic/Pharmacodynamic Models), human intake of 9.8 g of the congener over a 230-day period would result in peak concentrations of 720 and 2.1 ppm in fat and blood, respectively, 5 years after the onset of exposure. The model also predicted that the body burden after 5 years would be 5.2 g and the half-life 6.5 years. This half-life is shorter than the 12 years (median, range 4–97 years) calculated for hexabromobiphenyl in a Michigan cohort (Lambert et al. 1990) (see Section 3.8.1).

In rats fed diets containing octabromobiphenyl mixture for several weeks, adipose tissue and liver accumulated much more bromine than did skeletal muscle (Lee et al. 1975b). For example, after 2 weeks of treatment, adipose of rats dosed with 50 mg/kg/day had 200 times more bromine than did adipose of control rats; the liver of these rats had 100 times more bromine than the livers of controls. Feeding a PBB-free diet for 2 weeks decreased PBB levels in liver and muscle, but not in fat. Eighteen weeks after exposure, the concentration of bromine in the adipose tissue of rats dosed with 50 mg/kg/day continued to increase to . 840 times that of controls. Similar results were reported by Norris et al. (1975a, 1975b). These investigators also reported that 16 days after a single dose of octabromobiphenyl mixture in rats, PBB residues were present in the adrenals, adipose tissue, heart, and skin at levels ranging from 0.14 to 0.25% of the administered dose; the liver, pancreas, and spleen contained lesser amounts.

The distribution and elimination of PBBs from tissues were examined in rats over a period of 112 days after a single oral dose of FireMaster FF-1 (Domino et al. 1982). Elimination from blood was best described by a three-compartment model, and an elimination half-life from whole blood of 145 days was estimated. Relative to the three compartments (C1, C2, and C3): C1 consisted of heart, kidney, spleen, and whole blood; C2 included liver, lung, cerebrum, cerebellum, and testes; and C3 included subcutaneous fat. PBB residues in C1 rose quickly, peaked within 5 hours of dosing and then fell rapidly;

a half-life of 3.62 hours was estimated. PBB peaked in C2 at 12 hours and then decreased; the half-life was 17.6 hours. In C3, levels of PBB peaked only after 1 week and remained elevated for several weeks; the estimated half-life was 31.1 days. Tissues with PBBs in order of increasing concentration were: blood, spleen, kidney, and heart in C1, and testes, cerebrum, cerebellum, lung, and liver in C2. Simulations of different body fat proportions showed that reduction in body fat decreased the half-life of the chemical considerably. According to the investigators (Domino et al. 1982), tissues within C1 and C2 may be at greater risk of toxicity during the subacute phase of PBB ingestion. In their view, this could explain the fact that blood PBB levels in Michigan families were not positively correlated with toxic symptoms of exposure to PBBs (see Section 3.2).

In mink treated with FireMaster FF-1 in the diet for up to 11 months, the concentration of PBB residues in adipose tissue were higher than in brain and skeletal muscle at all times (Aulerich and Ringer 1979; Ringer et al. 1981). The source of the PBBs (FireMaster FF-1 versus food contaminated with PBBs) did not seem to have a significant influence on the qualitative or quantitative distribution of residues in tissues. Sows fed FireMaster BP-6 in the diet for 12 weeks also accumulated PBBs in adipose tissue; on a fat basis, the highest concentration of PBBs was found in the liver, followed by adipose, kidney, and brain (Werner and Sleight 1981). Distribution studies in guinea pigs after a single dose of FireMaster FF-1 showed preferential accumulation of residues in liver, kidneys, and lungs 2 days after dosing (Ecobichon et al. 1983). This was followed by a slow decrease in these organs, but levels in adipose tissue reached a maximum between 7 and 14 days after dosing and then decreased.

Several studies have examined the distribution of PBB residues in offspring after maternal exposure to PBBs during gestation and/or lactation. In 4-week-old pigs exposed *in utero* and via lactation to FireMaster BP-6, PBBs accumulated preferentially in adipose tissue and liver on a wet tissue basis. Over a wide range of doses, however, adipose had at least two times the PBB concentration compared to the liver (Werner and Sleight 1981). PBB levels in tissues of sows were comparable to those measured in tissues of 4-week-old pigs. On a fat basis, the liver had the highest concentration of PBBs in both sows and the young. In pigs exposed only *in utero*, PBB levels in liver and adipose were similar and considerably lower than in tissues of sows or 4-week-old pigs, suggesting that far more PBBs are transferred through lactation than through the placenta. A similar conclusion was reached in rat studies (McCormack and Hook 1982; Rickert et al. 1978). In contrast, PBB levels in liver and body fat of guinea pigs exposed briefly through lactation were considerably lower than the tissue levels acquired transplacentally in a 2-day period (Ecobichon et al. 1983). A biological half-life of 22 days in tissues of dams and pups was estimated in that study (Ecobichon et al. 1983).

Polybrominated Diphenyl Ethers. In male rats administered a single gavage dose of 1 mg/kg of a ^{14}C-labeled commercial octaBDE mixture, radioactivity could be detected on day 1 in all tissues taken from the rats (Norris et al. 1975b). Similar findings were reported for rats treated with a commercial decaBDE mixture, although the levels of radioactivity found in the tissues were lower than in the rats treated with octaBDE (Norris et al. 1975b). On day 16 after dosing, the adrenals, adipose tissue, heart, and skin of the octaBDE-dosed rats had 0.14–0.25% of the administered dose per gram of tissue; lesser amounts were detected in the liver, pancreas, and spleen. In contrast, on day 16 after dosing, only the adrenals and spleen from decaBDE-treated rats contained radioactivity. These findings are consistent with the poor absorption and rapid fecal elimination shown by the decaBDE compound.

Norris et al. (1975b) also examined the bromine content of tissues from rats administered either octaBDE or decaBDE (1 mg/kg/day) in the diet for 90 days followed by an additional 90-day period on a control diet. During the recovery period, interim sacrifices were conducted on days 0, 10, 30, and 60. On recovery day 0, kidney and serum from rats treated with either mixture had the same amounts of bromine as their respective controls. However, adipose tissue of octaBDE- and decaBDE-treated rats had approximately 90- and 4-fold more bromine per gram of tissue than their respective controls. These levels remained practically unchanged over the 90-day recovery period. In the liver, the bromine content from decaBDE-treated rats was elevated on day 0 of recovery, but decreased and stabilized near control values after day 10 of the recovery period. Bromine in liver from octaBDE-treated rats was increased approximately 8-fold relative to controls on day 0 and decreased gradually to about half that amount by day 90 of recovery.

In a study of rats treated for 180 days with decaBDE or octaBDE in the diet, bromine in the liver from decaBDE-dosed rats was slightly higher than in control rats throughout the study period, whereas octaBDE-treated rats showed a gradual and significant increase in liver bromine levels reaching about 7 times those of controls by the 180th day (Norris et al. 1975b). Analysis of adipose tissue showed an even more pronounced accumulation of bromine in octaBDE-dosed rats and practically no accumulation in decaBDE-dosed rats. No significant accumulation of bromine was seen in serum, kidney, skeletal muscle, or kidneys.

Studies conducted with analytical-grade decaBDE also showed poor absorption of the compound. Twenty-four hours after feeding rats ^{14}C-decaBDE most of the radiolabel (82–86%) was recovered in the feces and gut contents (El Dareer et al. 1987). No tissue sampled had more than 0.5% of the administered dose. In general, the highest amounts were found in the liver, and rats fed a lower dose retained more

label in the tissues than rats fed a higher dose. The latter suggests that the percent of the dose retained decreases as the amount of decaBDE in the diet increases.

Administration of a single gavage dose of ^{14}C-2,2',4,4'-5-pentaBDE to rats resulted in preferential deposition of label in the carcass (38%), adipose tissue (38.%), and blood (1.4%) 72 hours after dosing (Hakk et al. 1999). No other tissue had more than 1% of the radioactivity at 72 hours. Fractionation of the carcass showed that the majority of the label was in the skin. When deposition was expressed as concentration, the lipid-rich tissues such as adipose tissue, skin, and adrenals contained the highest concentration of radioactivity.

Analysis of tissues from rats 5 days after administration of a single gavage dose of ^{14}C-2,2',4,4'-tetraBDE showed that adipose tissue had an approximately 70 times higher concentration of label than other tissues on a fresh weight basis and 3.5-fold higher on a lipid weight basis (Örn and Klasson-Wehler 1998). The lungs had the second highest concentration of radiolabel with approximately twice that in the liver and kidneys. In mice treated in the same fashion, adipose tissues also had the highest concentration of radioactivity, 10-fold higher than other tissues on a fresh weight basis. On a lipid weight basis, adipose tissue had a concentration of ^{14}C similar to liver and twice that in lung and kidney.

Treatment of pregnant rats by gavage with decaBDE on Gds 6–15 resulted in no significant increase in bromine content in the liver of fetuses on gestation day 21 relative to controls (Norris et al. 1975b). However, placental transfer cannot be totally ruled out since no other fetal tissue was examined.

3.4.2.3 Dermal Exposure

Polybrominated Biphenyls. No studies were located regarding distribution of PBBs in humans after dermal exposure.

Increased liver weight and necrosis were observed in rabbits after application of an unspecified hexabromobiphenyl mixture to the skin, suggesting that PBBs or metabolites reached that organ (Waritz et al. 1977). No further information was available.

Polybrominated Diphenyl Ethers. No studies were located regarding distribution of PBDEs in humans or animals after dermal exposure.

3.4.2.4 Other Routes of Exposure

Polybrominated Biphenyls. In general, the distribution pattern of PBBs after parenteral administration is similar to that obtained after oral exposure. In rats, immediately after intravenous injection of ^{14}C-2,2',4,4',5,5'-hexabromobiphenyl adipose, skin, muscle, liver, and blood contained . 29, 20, 40, 10, and 1.5% of the dose, respectively (Matthews et al. 1977). Seven to 42 days postdosing, most of the residue in liver and muscle was redistributed to adipose tissue. The percent of the dose remaining in liver and muscle on day 42 was 0.8 and 3.5%, respectively. The concentration of radioactivity in skin remained relatively constant over a 42-day period. In a similar study in rats, the adipose/blood equilibrium distribution rate was found to be much higher than for any other tissue examined, and 4 days after dosing, adipose tissue contained $60% of the body burden (Tuey and Matthews 1980).

The elimination half-times from blood and several tissues were determined in rats administered a single intraperitoneal dose of 10 mg/kg FireMaster BP-6 (Miceli and Marks 1981). Elimination from serum followed first-order kinetics, and a half-time of 23.1 weeks was calculated over a 36-week period after dosing. Adrenal and adipose tissue had the highest PBB concentrations at week 6, and these levels were maintained throughout the 36-week observation period. Concentrations of PBBs were also elevated in the liver, lungs, and pituitary at week 6, whereas PBB levels in brain, kidney, and spleen were several-fold lower. Elimination half-times from adrenal, brain, fat, liver, lung, and spleen were 43.3, 63.0, 69.3, 11.5, 11.2, and 9.0 weeks, respectively. Elimination from heart, kidney, and pituitary did not appear to follow first-order kinetics; thus, elimination half-times from these tissues were not calculated. The concentration of PBB in adipose tissue was at least 4 times higher than in any other tissue, and unlike other tissues,

continued to increase, reaching a maximum at week 12 postdosing. The adipose/serum ratio of PBB concentration increased from 222 at 6 weeks to 722 at 36 weeks, reflecting the much more rapid elimination of PBB from serum than from adipose tissue. The investigators estimated that, given the elimination half-time from fat of 69 weeks, >1 μg/g of PBB would remain in fat by the time the rats reached 2 years of age, the end of their lifespans.

The distribution of PBB residues was also examined in pregnant mink and ferrets after injection of a mixture of 2,2'4,4',5,5'-hexabromobiphenyl and 2,2',3,4,4',5,5'-heptabromobiphenyl (Bleavins et al. 1980). Two hours after a single injection in the jugular vein on gestation day 37, the liver, kidney, and adipose tissue of ferret dams had 1.625, 0.108, and 0.124% of the dose/g tissue, respectively. PBB levels in fetal tissues did not exceed 0.013% of the dose (liver). In mink, PBB levels in maternal liver, kidney, and adipose tissue were 1.622, 0.087, and 0.031% of the dose/g tissue, respectively. Fetal liver had the highest amount of PBBs, 0.005% of the dose. In a different experimental series, the investigators (Bleavins et al. 1980) also showed that the dam's milk was the major route of offspring exposure since PBB levels, on a per gram or per kit basis, were significantly higher in 2-week-old kits than in newborn kits. The ratio of the 2-week PBB concentration to the birth concentration was 3.94/g and 36.66/kit. On a per kit basis, treated newborn kits accumulated 0.80% of the maternal dose through *in utero* exposure.

Polybrominated Diphenyl Ethers. No relevant information was located regarding distribution of PBDEs following exposure by non-natural routes of administration.

3.4.3 Metabolism

Polybrominated Biphenyls. Information regarding the metabolism of PBBs in humans is limited. Chromatographic analysis of serum samples from Michigan dairy farmers and from Michigan Chemical Corporation employees revealed some differences in peak profile between these two groups and between these two groups and the peak profile of FireMaster BP-6 (Wolff and Aubrey 1978; Wolff et al. 1979a). Farmers had a lower concentration of two pentabromobiphenyls relative to chemical workers and FireMaster BP-6. Both farmers and workers had a significant reduction in the amount of 2,2',3,4,4',5,5'-heptabromobiphenyl relative to FireMaster BP-6. Other minor differences between the groups were also apparent. The differences in peak profiles between farmers and chemical workers were attributed to different routes of exposure. Farmers had predominantly dietary exposure to PBBs which, according to the authors (Wolff and Aubrey 1978), could have undergone partial metabolism in the animal food source (see below). It should be noted that chemical transformation of the PBBs due to

cooking of meat or pasteurization of milk should not be expected since the temperatures reached during these processes is probably not high enough. As discussed in Chapter 4, temperatures must exceed .500 EC for structural alterations of PBBs to occur. Nevertheless, a significant reduction (36–52%) in the concentration of PBBs in pressure-cooked meat relative to raw meat (due to loss of fat) has been reported (Zabik et al. 1978). The decreased heptabromobiphenyl peak in farmers and workers relative to FireMaster BP-6 may reflect poor absorption of this congener since it is not expected to be metabolized readily (Wolff and Aubrey 1978; Wolff et al. 1979a).

Human exposure to PBBs in the Michigan contamination episode occurred primarily through consumption of contaminated meat and dairy products. The limited information available regarding the metabolism of PBBs in dairy cattle is insufficient to ascertain whether humans ingested PBBs or metabolic products of PBBs. In a controlled study, cows fed single or repeated doses of FireMaster BP-6 excreted 50% of the dose in the feces as parent compound (Willet and Durst 1978). Tissues, feces, or urine were not analyzed for metabolites. Results of studies in rats, and also *in vitro* studies (see below), have shown that highly brominated PBB congeners, such as the major components of FireMaster BP-6, undergo little or no metabolic transformation. Based on the existing information, it seems reasonable to assume that in the Michigan contamination episode, humans consumed mainly unchanged penta-, hexa-, and heptabromobiphenyls.

The *in vivo* metabolism of some PBB congeners and of commercial PBB mixtures has been investigated in a limited number of animal studies. For example, in pigs, intraperitoneal injection of 4-bromobiphenyl yielded three urinary metabolites: 4'-bromo-4-biphenylol (3% of the dose), bromobiphenylol (traces), and 4'-bromobiphenylol (0.5% of the dose) (Kohli and Safe 1976). 4,4'-Dibromobiphenyl yielded four urinary metabolites: 4,4'-dibromo-3-biphenylol (5% of the dose), 3,4'-dibromo-4-biphenylol (1% of the dose), 4'-bromo-3-methoxy-4-biphenylol (1% of the dose), and traces of a dibromomethoxybiphenyl. The authors suggested these results indicate that metabolism of the 4,4'-congener occurs through the formation of an arene oxide. The major urinary metabolite of FireMaster BP-6 was a pentabromobiphenylol (1% of the dose), which could have resulted from direct hydroxylation of the minor pentabromobiphenylol isomers in FireMaster BP-6 or by debromination/hydroxylation of the major congener, 2,2',4,4',5,5'-hexabromobiphenyl.

In rabbits, metabolism of 2-bromobiphenyl yielded two polar metabolites, one metabolite was identified as 2'-bromo-4-biphenylol (1% of the dose), and the other metabolite (traces) was also a monohydroxylated derivative, but the position of the hydroxyl group was not determined (Kohli et al.

1978). 3-Bromobiphenyl produced a major metabolite (4% of the dose) identified as either 3-bromo-4-biphenylol or 5-bromo-2-biphenylol; a minor dihydroxylated metabolite was also detected. 4-Bromobiphenyl yielded two metabolites: 4'-bromo-4-biphenylol (2% of the dose) and 4'-bromo-3,4-biphenyldiol (1.5% of the dose). Experiments with tritiated 4-bromobiphenyl suggest that the metabolism of this congener involves the formation of an arene oxide.

Similar results have been reported in rats (Sparling et al. 1980). 4'-Bromo-4-biphenylol was the major metabolite of 4-bromobiphenyl. 2-Bromobiphenyl was metabolized to 2-bromo-4,4'-biphenyldiol and 2-bromo-4',5-biphenyldiol; 2-bromo-5-biphenylol was a minor metabolite. 3-Bromobiphenyl also yielded diols as major metabolites: 3-bromo-4,4'-biphenyldiol and an unknown diol. The main conclusions of this experiment were: the major site of hydroxylation is at the *para* position of the unsubstituted phenyl ring, and also at the *para* position of the ring for the 2- and 3-brominated congeners; substitutions in positions 2 and 3 tend to direct hydroxylation to position *para* and *ortho* (minor) to the substituents; and the 2- and 3-hydroxylated products are subsequently dehydroxylated, whereas the 4'-hydroxy congener is not.

In contrast to the lower brominated congeners, no major metabolites were identified in the urine or feces of rats treated with a single intraperitoneal dose of 2,2'4,4'5,5'-hexabromobiphenyl, suggesting that this congener is stable and persistent (Safe et al. 1978). Analyses of the feces of dogs administered FireMaster BP-6 orally revealed the presence of a metabolite identified as 6-hydroxy-2,2',4,4',5,5'-hexabromobiphenyl (Gardner et al. 1979). This metabolite was not found in the liver, but the parent compound was identified. Since hydroxylation in position 6 of highly substituted congeners is unlikely, it was postulated that the metabolite found in the feces was formed by microbial metabolism of the PBB in the intestinal tract. The *in vitro* metabolism of 2,2',4,4',5,5'-hexabromobiphenyl with liver microsomes of rats induced with either 2,2',4,4',5,5'-hexabromobiphenyl or FireMaster BP-6 produced three major metabolic fractions: lipophilic ether soluble polar metabolites, trichloroacetic acid (TCA) soluble conjugates, and macromolecular adducts (Purdy and Safe 1980).

The NADPH-dependent metabolism of a PBB mixture was studied *in vitro* with liver microsomes of rats induced with PB, PBB, or 3-MC (Dannan et al. 1978a). Of the 12 major components of the mixture, only 2,2',4,5,5'-pentabromobiphenyl and a hexabromobiphenyl were metabolized by microsomes from PB- or PBB-treated rats. Of seven structurally identified PBB components, only 2,2',4,5,5'-pentabromobiphenyl had a bromine-free *para* position. Although 2,2',4,5,5'-pentabromobiphenyl, 2,3',4,4',5-pentabromobiphenyl, and 2,2',3,4,4',5'-hexabromobiphenyl have two adjacent unsubstituted carbons, only

2,2',4,5,5'-pentabromobiphenyl was metabolized. No significant metabolism occurred when the PBB mixture was incubated with microsomes of control rats or MC-induced rats. When 2,2'- and 4,4'-dibromobiphenyl were incubated with liver microsomes of PB-treated rats, only the 2,2'-congener was metabolized. These results suggest that the presence of a free *para* position is required for the metabolism of brominated biphenyls and that the bromine content of the molecule is less important in determining metabolism than the position of bromines on the biphenyl nucleus.

A more recent study with hepatic microsomes of induced rats showed that MC pretreatment increased the NADPH-dependent metabolism of PBB congeners (di-, tri-, and tetrabrominated), which had adjacent unsubstituted *ortho* and *meta* positions on at least one ring (Mills et al. 1985). Some penta- and hexabromobiphenyls that have adjacent unsubstituted *ortho* and *meta* positions were not metabolized, suggesting that further bromination prevents metabolism. Pretreatment with PB increased the microsomal metabolism of congeners that have adjacent unsubstituted *meta* and *para* positions on at least one ring. It was concluded that the rates of metabolism of PBB congeners depends on the position of the bromines and the form of the cytochrome P-450 induced. The ability to metabolize PBBs also depends on the species. For example, hepatic microsomes isolated from rats have a greater potential to metabolize PBBs than hepatic microsomes isolated from pigeons (Borlakoglu and Wilkins 1993).

Polybrominated Diphenyl Ethers. Three main metabolic peaks were identified in the feces from rats fed reagent-grade decaBDE for 8 days (El Dareer et al. 1987). Of the total decaBDE-derived radioactivity recovered in the feces, 1.5–27.9% constituted metabolites. Since absorption of decaBDE is minimal, El Dareer et al. (1987) suggested that metabolism apparently took place in the gastrointestinal tract. In parallel experiments in rats injected ^{14}C-decaBDE intravenously, feces and gut contents contained 74% of the administered radioactivity 72 hours after the injection, and 63% of the excreted material was metabolites. In rats dosed intravenously with ^{14}C-decaBDE and with biliary cannulas, 7.17% of the dose appeared in the bile, and <1% of this amount was unchanged decaBDE. Only one metabolite was detected. The structure of the metabolites detected in these experiments was not defined.

Analysis of fecal extracts of rats administered a single gavage dose of ^{14}C-2,2',4,4',5-pentaBDE showed, after methylation, the presence of two monomethoxy pentaBDE metabolites and two de-brominated monomethoxy tetraaBDE metabolites (Hakk et al. 1999); however, the precise structure of these metabolites could not be determined. Less than 10% of the radiolabel was found in these metabolites and >90% was present as parent compound. In rats with cannulated biliary ducts, the investigators identified two monohydroxypentaBDE metabolites and two dihydroxypentaBDE metabolites. No glucuronide or

sulfate conjugates were found in the bile. There was some evidence suggesting the presence of a thio-substituted pentaBDE possibly formed through the mercapturic acid pathway.

Five metabolites were detected in the feces of rats and mice collected over a 5-day period after administration of a single dose of ^{14}C-2,2',4,4'-tetraBDE (Örn and Klasson-Wehler 1998). Although the precise structure was not identified, the data suggested that the metabolites were hydroxylated derivatives of tetraBDE. An additional metabolite, possibly a methyl thio-substituted tetraBDE, was also detected in the feces. Yet, at least 85% of the radioactivity in the feces corresponded to the parent compound. A water-soluble metabolite was detected in urine from mice, but could not be isolated. Unchanged tetraBDE was also the major compound in all tissues analyzed, but minor amounts of hydroxylated tetraBDE metabolites were detected.

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

No studies were located regarding excretion of PBBs or PBDEs in humans or animals after inhalation exposure.

3.4.4.2 Oral Exposure

Polybrominated Biphenyls. No studies were located that provide information on percentage of ingested PBBs excreted by humans. However, PBBs in biliary fluid of a group of farmers and chemical workers ranged from undetected to 70 µg/L, and the correlation between serum PBB levels and levels in bile was statistically significant (Eyster et al. 1983). Similarly, PBB levels in feces ranged from undetected to 862 µg/kg, and the correlation between serum PBB levels and fecal levels was also statistically significant (Eyster et al. 1983).

Serum half-life values have been estimated using human data from the Michigan PBB cohort (Lambert et al. 1990; Michels Blanck et al. 2000b; Rosen et al. 1995). A median half-life of 12.0 years (95% CI 4–97 years) was estimated based on two serum measurements from 15 women ($20 years of age) with an initial serum PBB level of $5 ppb (Lambert et al. 1990). An analysis of 51 women ($18.8 years of age) and 112 men ($18.1 years of age) with at least two measurements 1 year apart and an initial PBB level of $20 ppb found a median half-life of 13.0 years (95% CI 6.3–infinite years) and 10.0 years (95% CI 6.7–20.0 years), respectively (Rosen et al. 1995). Based on a median half-life of 10.8 years (95% CI 9.2–14.7 years) for the entire group (163 persons, median PBB level 45.5 ppb), it was estimated that it will take more than 60 years for their PBB levels to fall below a detection limit of 1 ppb.

Determinants of PBB serum decay were investigated in 380 Michigan women ($16 years of age) who had an initial PBB level of at least 2 ppb and at least two measurements taken when they were not pregnant (Michels Blanck et al. 2000b). The mean initial PBB level was 20.9 ppb (standard deviation 78.7), and the mean time between the first and last measurement was 4.2 years (range 16.0–75.2 years). A total of 109 women (29%) did not have a reduction in serum PBBs over time. Assuming that PBBs reached equilibrium in the body before substantial amounts were eliminated and before the first serum measurements were taken, the entire body was modeled as a single compartment for PBBs with exponential decay. The median PBB half-life in the entire group was 13.5 years (95% CI 10.5–23.2 years). Subject-specific decay rate estimates were regressed on predictor variables including initial age, body mass index (BMI), smoking history, breast-feeding duration, and parity. The serum PBB decay rate was slower, resulting in a longer half-life, with higher initial PBB levels; women with initial PBB levels of <10 and >10 ppb had median half-lives of 12.9 and 28.7 years, respectively. The PBB decay rate was also slower (p=0.03) in women with an initial BMI above the median (BMI$23). Increasing number of pregnancies between the first and last measurement was also associated with slower decay, but the effect was of borderline statistical significance (p=0.06). Breast feeding as either a

continuous variable or as categorized by tertiles of duration (<3, 3–9, or >9 months), age, and smoking were not associated with serum PBB decay.

Lactation constitutes the most important route of excretion of PBB in lactating women. Numerous studies reported PBB levels in breast milk from Michigan women (Brilliant et al. 1978; Eyster et al. 1983; Humphrey and Hayner 1975; Jacobson et al. 1984; Landrigan et al. 1979). PBB levels in breast milk on a lipid basis ranged from undetected to 92,667 µg/kg, with a median of 250 µg/kg, in a group of parturient women from Michigan (Eyster et al. 1983). Regression analysis of the data revealed that on a lipid basis, PBBs are 107–119 times more concentrated in milk than in serum. Also, adipose PBB levels are 1.1–1.5 times higher than the breast milk levels when milk levels were $100 µg/kg.

The importance of PBB transfer through lactation in experimental animals is discussed in Section 3.4.2.2.

There is limited information regarding excretion of PBBs in experimental animals. Rats dosed once with ^{14}C-2,2',4,4',5,5'-hexabromobiphenyl excreted 7.9% of the dose in the feces within the first 24 hours; urinary excretion data were not provided (Matthews et al. 1977). It was estimated that <10% of the administered dose would ever be excreted. These results are consistent with those of other investigators who report that this congener is stable and persistent (Safe et al. 1978). In rats gavaged with ^{14}C-2,2',4,4',5,5'-hexabromobiphenyl for 22 days, between 10 and 20% of the daily dose was excreted daily in the feces; this value was predominantly due to elimination of unabsorbed PBB (Tuey and Matthews 1980). In monkeys, the main route of excretion of hexabromobiphenyl residues was also in the feces (Rozman et al. 1982). Between 60 and 70% of the administered dose was excreted in the feces in the first 11 days after dosing; urinary excretion was minimal. The difference between the absorption rate reported by Matthews et al. (1977) and that reported by Rozman et al. (1982) can probably be accounted for by differences in the experimental designs.

Rats treated with a single gavage dose of ^{14}C-octabromobiphenyl excreted <1% of the administered dose in urine and expired air over a 16-day period (Norris et al. 1975a). Within the first 24 hours after dosing, 61.9% of the dose was present in the feces. The proportion that represents unabsorbed PBB is not known. By day 16, 74% of the administered dose had been recovered in the feces.

Polybrominated Diphenyl Ethers. Elimination half-lives for components of a commercial pentaBDE mixture (Bromkal 70) from perirenal fat of rats were estimated to be between 19 and 111 days (von Meyerinck et al. 1990). The rats were administered a single gavage dose of 300 mg/kg of the mixture and

analyses of perirenal fat were conducted for up to 70 days after dosing. Analysis of the mixture, revealed at least seven components: one tetraBDE, three pentaBDEs, and three hexaBDEs, but the stereochemistry of the individual compounds was not ascertained. In general, the elimination half-lives increased as the degree of bromination increased. Also, no significant differences were observed between male and female rats with the exception of the tetraBDE, which exhibited a significantly higher elimination half-life in female rats.

In rats treated by gavage with a single 1 mg/kg dose of commercial ^{14}C-decaBDE or octaBDE mixtures, most of the excreted radioactivity measured over a 16-day period was in the feces and <1% of the label was detected in the urine and expired air (Norris et al. 1975b). No significant differences in excretion patterns were observed between males and females, but the excretion rate of decaBDE was very different than that of octaBDE. Approximately 91% of the decaBDE-derived radioactivity was found in the feces in the first 24 hours and almost all radioactivity (>99%) was accounted for by day 2 after dosing. In contrast, approximately 62% of the administered radioactivity derived from octaBDE was detected in the feces after the first 24 hours, after which time, an additional 11% of the label was slowly eliminated over the next 15 days. At the end of the study period, 26.4% of the octaBDE-derived radioactivity had not been recovered in the excreta. The investigators estimated an elimination half-life of <24 hours for decaBDE; octaBDE had an initial phase half-life of <24 hours and a second phase half-life of >16 days. A more recent study with ^{14}C-decaBDE in rats found that >9% of the dose was excreted in the bile over a 72-hour period following dosing compared to #0.02% in the urine (Hakk et al. 2000).

In an acute feeding study in rats fed analytical-grade decaBDE for 12 days and ^{14}C-decaBDE on day 8, recovery of radioactivity in the feces on days 9–12 ranged from 91.3 to 101% of the amount ingested (El Dareer et al. 1987).

The feces was the major route of elimination of 2,2',4,4',5-pentaBDE in rats administered a single dose of the ^{14}C-labeled compound (Hakk et al. 1999). Over a 72-hour period, <1% of the administered dose was recovered in the urine and approximately 42% in the feces. In rats with cannulated bile ducts, <0.4% was detected in the urine and about 87% in the feces over the same period of time.

Rats given a single gavage dose of 14.6 mg/kg of ^{14}C-2,2',4,4'-tetraBDE excreted 14% of the dose in the feces and <0.5% in the urine over a five day period (Örn and Klasson-Wehler 1998). Mice treated in the same manner excreted 20% of the dose in the feces and 33% in the urine over the same time period. The amount of non-absorbed material in the feces on day 1 was approximately 5 and 7% of the dose in rats

and mice, respectively, suggesting efficient absorption in both species (Örn and Klasson-Wehler 1998). The results also suggest that mice have a more efficient metabolism and excretion of tetraBDE than rats.

3.4.4.3 Dermal Exposure

No studies were located regarding excretion of PBBs or PBDEs in humans or animals after dermal exposure.

3.4.4.4 Other Routes of Exposure

Polybrominated Biphenyls. Rats given a single intravenous dose of ^{14}C-2,2',4,4',5,5'-hexabromobiphenyl excreted a cumulative 0.96, 3.3, and 6.6% of the dose in the feces 1, 7, and 42 days after dosing, respectively (Matthews et al. 1977). Only traces (0.1% of the dose) were excreted in the urine. Two decay components were calculated from excretion data; an initial decay rate of 1.05% of the dose/day and a later rate of 0.15% of the dose/day. Biliary excretion accounted for 0.68% of the dose between 0 and 4 hours after dosing. Analysis of bile and feces showed that at least 95% of the radioactivity corresponded to the parent compound. Moreover, in rats, . 35% of the radioactivity excreted in the bile during the first week after a single dosing was reabsorbed (Tuey and Matthews 1980).

Parenteral administration of mono- and dibromobiphenyls to rats, rabbits, and pigs suggests that the urine is an important route of excretion for polar metabolites (Kohli and Safe 1976; Kohli et al. 1978; Sparling et al. 1980). However, cumulative urinary excretion did not account for more than 5% of the administered doses. Data regarding fecal excretion were not provided.

Polybrominated Diphenyl Ethers. No relevant information was located regarding elimination of PBDEs following exposure by non-natural routes of administration.

3.4.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen et al. 1987; Andersen and Krishnan 1994). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations in risk assessment than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parametrization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

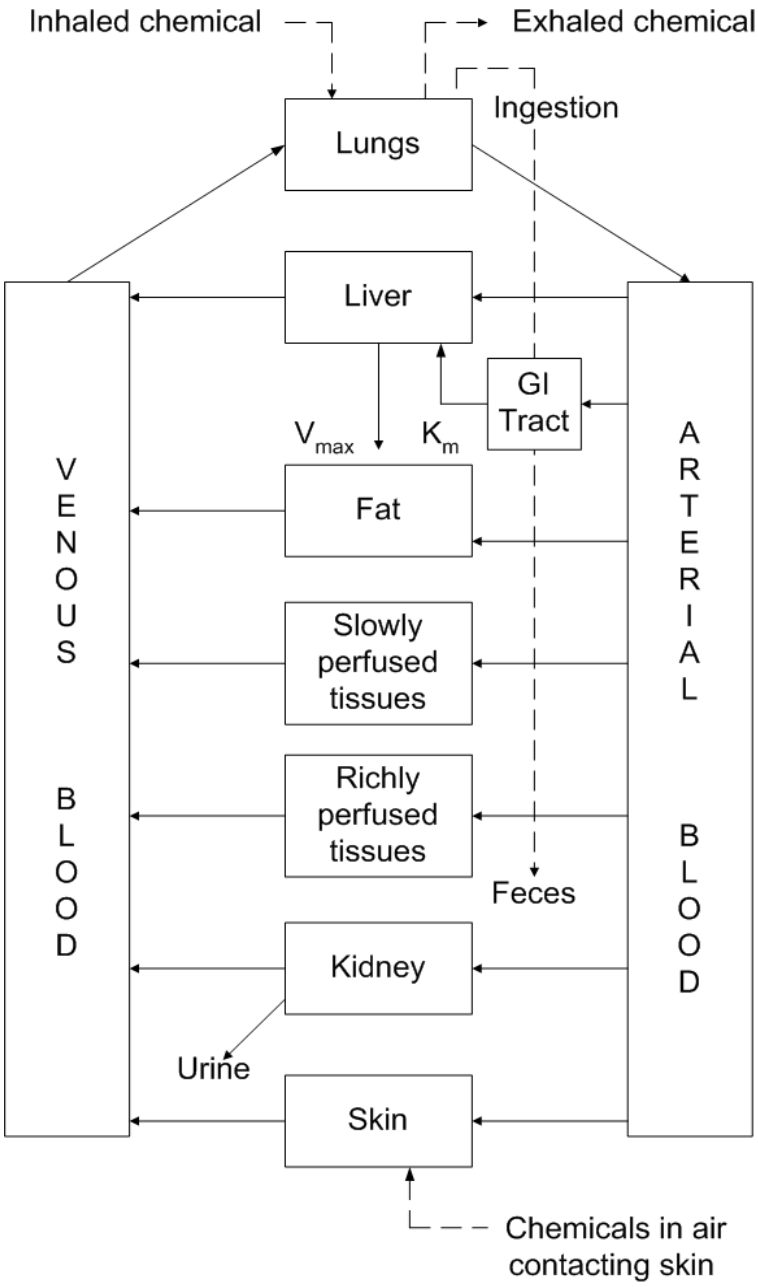
The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) is adequately described, however, this simplification is desirable because data are often unavailable for

many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. PBPK models also provide a basis for extrapolating better toxicity values for use in human risk assessment. Figure 3-3 shows a conceptualized representation of a PBPK model.

Polybrominated Biphenyls. A PBPK model that incorporates tissue volume, affinity for PBBs, and rate of perfusion was developed to describe the distribution and body burden of the major component of FireMaster PBB mixtures, 2,2',4,4',5,5'-hexabromobiphenyl, in the rat (Tuey and Matthews 1980). The modeling methods are an extension of those used to predict the disposition of PCBs (Matthews et al. 1977). The model predicts that at equilibrium, changes in the PBB concentration or changes in tissue volume of any tissue will lead to a corresponding change in all tissues. For example, if the concentration of a PBB congener in the liver is reduced by metabolism or excretion, then the concentration of that PBB congener in all tissues will be reduced proportionally. Congeners that cannot be readily metabolized (as is the case for 2,2',4,4',5,5'-hexabromobiphenyl) or excreted will concentrate in adipose tissue, but will still circulate to other tissues. Exposure to other tissues will be proportional to the respective tissue/blood ratios and the concentration in main storage tissues. This dynamic distribution results in accumulation of persistent congeners in all tissues and depletion from all tissues of those congeners that can be cleared. In rats orally dosed daily with ^{14}C-2,2',4,4',5,5'-hexabromobiphenyl over a 30-day period, tissue concentrations on day 31 were (in increasing order): blood, muscle, liver, skin, and adipose, and were in general agreement with those predicted by the PBPK model. When the model was scaled to nonlactating humans by adjusting for tissue volume, blood flow, and clearance and rate constant parameters, human intake of 9.8 g of the congener from milk consumption over a 230-day period would result in peak concentrations of 720 and 2.1 ppm in fat and blood, respectively. The model also predicted that the body burden after 5 years would be 5.2 g and the half-life 6.5 years. The half-life of 6.5 years predicted using the rat-based PBPK model is shorter than half-life values estimated using human sera

Figure 3-3. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Source: adapted from Krishnan et al. 1994

Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

data from the Michigan PBB cohort (Lambert et al. 1990; Michels Blanck et al. 2000b; Rosen et al. 1995), as discussed in Section 3.4.4 (Elimination and Excretion).

Polybrominated Diphenyl Ethers. No PBPK/PD models were located for PBDEs.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Polybrominated Biphenyls. The mechanism by which PBBs enter the blood stream from the lungs, skin, or gastrointestinal tract is not known and little information is available on how PBBs are distributed in the body. The available data indicate that the absorption mechanism is likely passive diffusion. Results from studies of Michigan subjects showed that in the blood stream, 80% of the PBBs was bound to protein and 20% was associated with lipids (Greaves et al. 1984). Of the fraction bound to protein, 73% was bound to apolipoprotein B and the remaining percent was bound to apolipoprotein A. In an *in vitro* model, shown to be representative of environmentally contaminated blood, the distribution of PBBs among plasma, erythrocytes, mononucleocytes, and polymorphonucleocytes was 89:9:<1:<1, respectively (Roboz et al. 1985).

In an *in vitro* study in an adipocyte cell line (3T3L1 cells), >75% of the 2,2',4,4',5,5'-hexabromobiphenyl taken up by the cells was associated with subcellular fractions that contained 85% of the cellular triglyceride, with only 20% of the compound found in the microsomal plasma-membrane fraction (Kraus and Bernstein 1986). This study also found that inhibition of respiration by cyanide at a concentration that completely inhibited oxygen consumption did not affect uptake of 2,2',4,4',5,5'-hexabromobiphenyl, supporting the assumption that because of their lipophilic nature, PBBs penetrate membranes by passive diffusion.

Polybrominated Diphenyl Ethers. No studies were located regarding pharmacokinetic mechanisms for PBDEs.

3.5.2 Mechanisms of Toxicity

PBBs and PBDEs share some toxicological properties with other structurally similar compounds, particularly PCBs, chlorinated dibenzo-*p*-dioxins (CDDs), and chlorinated dibenzofurans (CDFs) (Agency for Toxic Substances and Disease Registry 1994, 1998, 2000). However, for PBDEs, the introduction of the ether bridge makes the classification of congeners as either "dioxin-like" or "nondioxin-like" less unequivocal. Studies of structure-induction properties, structure-affinity binding properties, and structure-toxicity (with polychlorinated diphenyl ethers [PCDEs]) properties suggest that some *ortho* substituted PBDE (or PCDE) congeners can exhibit stronger affinity for the Ah receptor and exhibit stronger dioxin-type toxicity than their corresponding non-*ortho* substituted analogs (Chen et al. 2001; Howie et al. 1990). For example, 3,3',4,4'-tetraCDE was less immunotoxic and a less potent inducer of EROD and aryl hydrocarbon hydroxylase (AHH) than 2,3',4,4',5-pentaCDE (Howie et al. 1990). This is contrary to what would have been expected for the corresponding PCB or PBB congeners. This has been attributed to the greater distance between the two biphenyl rings in PBDE or PCDE congeners, relative to PCBs or PBBs. In other words, introduction of *ortho* substitutions into PBDEs or PCDEs does not create a spatial impediment for the two phenyl rings to assume a semi-flat position respect to each other as it does for PCBs or PBBs. This has implications not only for dioxin-type toxicities, but also for nondioxin-type toxicities. For example, studies have shown that mono- and diortho-substituted PCBs exhibit neurotoxic properties and structure-activity relationships for various neurological end points have been established (see Agency for Toxic Substances and Disease Registry 2000 for details). Although structure-activity relationships have not yet been fully examined for PBBs or PBDEs, it is reasonable to speculate that mono- and diortho-substituted PBDEs may not necessarily follow the neurotoxic potency rankings constructed with mono- and diortho-substituted PCBs.

There are also geometrical differences in PCBs, PBBs, and PBDEs due to the higher atomic weight and considerably larger molecular volume of bromine compare to chorine (Hardy 2000, 2002). These differences contribute to dissimilar physical/chemical properties that can influence the relative bioavailability, absorption, tissue accumulation, receptor interactions, and toxicities of the chemicals. For example, a comparison of a series of isosteric 3,3',4,4'-tetrahalobiphenyls in rats showed that relative toxicity (growth rate and thymic atrophy), Ah receptor binding affinity, and AHH and EROD induction potencies increased with increasing bromine substitution (Andres et al. 1983). Possible explanations for this effect included the increased polarizability of bromine versus chlorine and differences in the electronic, hydrophobic, and hydrogen bonding characteristics of bromine and chlorine (Andres et al. 1983).

Polybrominated Biphenyls. The mechanism of toxicity for PBBs has been extensively studied, but is not completely understood (Akoso et al. 1982a, 1982b; Andres et al. 1983; Dannan et al. 1982a, 1982b; Goldstein et al. 1979; Parkinson et al. 1983; Render et al. 1982; Robertson et al. 1982; Safe 1984). Many PBBs, PCBs, chlorinated dibenzo-*p*-dioxins (CDDs), chlorinated dibenzofurans (CDFs), and other structurally related halogenated aromatic hydrocarbons are believed to share a common mechanism of action strongly related to similarities in their structural configuration. Most of what is known regarding the mechanism of action of these compounds is based on structure-receptor binding relationships, structure-induction relationships, and structure-toxicity relationships (Goldstein and Safe 1989; Safe 1990). Most of the studies providing this information used parenteral routes of exposure and/or *in vitro* test systems, as explained below. It is beyond the scope of this profile to discuss these studies in detail.

A limited number of studies have shown that some PBB congeners bind to a cellular receptor (Ah [aryl hydrocarbon] receptor), which regulates the synthesis of a variety of proteins. This receptor was identified in the cytosol of mouse liver cells (Poland et al. 1976) and, subsequently, in extrahepatic tissues of laboratory animals, mammalian cell cultures, and human organs and cell cultures (Goldstein and Safe 1989). The structure-binding relationships for the coplanar 3,3',4,4',5-pentabromobiphenyl, the monoortho substituted congener 2,3,3',4,4',5-hexabromobiphenyl, and the diortho substituted analog 2,2,'5,5'-tetrabromobiphenyl were examined in rat liver cytosol (Safe et al. 1985). At PBB concentrations 1,000-fold (10 μM) greater than tetrachlorordibenzo-*p*-dioxin (TCDD) concentrations (10 nM), the coplanar congener completely displaced radiolabeled 2,3,7,8-TCDD from the cytosolic Ah receptor protein, the monoortho analog partially displaced the radiolabel, and 2,2,'5,5'-tetrabromobiphenyl was the least active competitor. The latter congener is relatively nontoxic and does not induce AHH. The Ah-binding characteristics of 3,3',4,4'-tetrabromobiphenyl and 3,3'4,4'5,5'-hexabromobiphenyl, both coplanar, were also examined in rat and mice liver cytosol (Millis et al. 1985). The results showed than the tetrabromobiphenyl was 10 times more effective than the hexabromobiphenyl in displacing radiolabeled 2,3,7,8-TCDD from the receptor. The stereospecific nature of the binding (high affinity seen with congeners substituted in both *para* and two or more *meta* positions) strongly suggests that a biological receptor mediates the responses caused by some PBBs.

The ability of PBBs to induce hepatic Phase I xenobiotic metabolizing enzymes (cytochrome P-450-dependent monooxygenases) is well documented (Dannan et al. 1978b, 1982a, 1982b, 1983; Ecobichon et al. 1979; Moore et al. 1978, 1979; Parkinson et al. 1983; Robertson et al. 1982; Schramm et al. 1985). PBB mixtures were classified as "mixed-type" inducers of hepatic microsomal monooxygenases and resembled a mixture of phenobarbital (PB)-like plus 3-methylcholanthrene (MC) as inducers of P-450

isozymes from CYP1A and CYP2B families. The CYP1A1 and CYP1A2 genes are induced by Ah receptor agonists, such as 2,3,7,8-TCDD and MC, and the structure-induction relationships for PBBs as inducers of these P-450 isozymes and their related activities have also been determined (Dannan et al. 1983; Parkinson et al. 1983; Robertson et al. 1982). For example, when injected intraperitoneally to immature male Wistar rats, the coplanar derivatives, 3,4,4'-tri-, 3,4,4',5-tetra-, 3,3',4,4'-tetra-, 3,3',4,4'5-penta-, and 3,3',4,4',5,5'-hexabromobiphenyl had a pattern of induction resembling that of MC (Robertson et al. 1982). Similar type experiments have shown that monoortho-bromo-substituted analogs of the coplanar PBBs, such as 2,3',4,4'-tetra-, 2,3',4,4',5-penta-, and 2,3',4,4',5,5'-hexabromobiphenyl, exhibit a mixed-type induction activity and resemble FireMaster BP-6 in their mode of induction (Dannan et al. 1978b; Parkinson et al. 1983). Yet a third group of PBB congeners, the diortho-bromo analogs of the coplanar PBBs, resemble PB in their mode of induction (PB induces the CYP2B1 and CYP2B2 genes). Among the diortho-bromo-substituted PBBs studied are 2,2,'5,5'-tetra-, 2,2'4,5,5'-penta-, 2,2',4,4',5,5'-hexa-, and 2,2',3,4,4',5,5'-heptabromobiphenyl (Moore et al. 1979; Parkinson et al. 1983). Results from studies with some dibromobiphenyls revealed that 4,4'-dibromobiphenyl resembled PB in its mode of induction (Robertson et al. 1982), whereas 2,2'-dibromobiphenyl had no significant effect on hepatic microsomal drug-metabolizing enzymes (Moore et al. 1979). The results of these experiments indicated that coplanar PBB congeners substituted in both *para* and one or more *meta* positions are MC-type inducers, diortho substituted congeners are PB-type inducers, and monoortho analogs of the coplanar PBBs are mixed-type inducers. These results were qualitatively similar to those obtained with PCBs and support the idea of a common receptor-mediated mechanism of action for PBBs. PBBs are also efficacious inducers of hepatic phase II metabolizing enzymes such as glutathione transferases, UDP glucuronyl transferases, and epoxide hydrolase (Parkinson et al. 1983; Schramm et al. 1985). For example, when intraperitoneally injected in rats, FireMaster BP-6 efficaciously induced hepatic glutathione transferases while concomitantly depressing selenium-dependent glutathione perioxidase activity, an important antioxidant enzyme in the liver (Schramm et al. 1985).

Many studies that examined structure-induction relationships for several PBB congeners also studied structure-toxicity relationships. Thymus and spleen weight were significantly reduced in rats by a series of MC-type inducers (Robertson et al. 1982). Further experiments in rats revealed that of a series of PBB congeners, only MC-type inducers significantly decreased thymus weight and body weight; PB-type, mixed-type, and MC-type inducers increased relative liver weight (Parkinson et al. 1983). Results from feeding studies in rats indicate that 3,3',4,4',5,5'-hexabromobiphenyl (MC-type) and 2,2',4,4',5,5'-hexa-bromobiphenyl (PB-type) increased liver weight; however, only the MC-type inducer decreased body weight and thymic and splenic weight, and caused lymphocytic depletion in the thymus (Render et al.

1982). Similar results were obtained when the toxicities of 3,3',4,4'-tetrabromobiphenyl (MC-type) and 2,2'5,5'-tetrabromobiphenyl (weak PB-type) were compared in rats (Robertson et al. 1983a). Only 3,3',4,4'-tetrabromobiphenyl caused significant reductions in growth rate and thymus size and marked depletion of lymphocytes from the thymic cortex. Results from studies with FireMaster BP-6 revealed that the pattern of toxic responses and the magnitude of the responses attributed to this mixture are consistent with it being composed of both MC-type and PB-type congeners; the most toxic responses being attributed to the MC-type congeners (Akoso et al. 1982a, 1982b; Dannan et al. 1982a, 1982b; Ecobichon et al. 1979; Parkinson et al. 1983; Render et al. 1982). These results suggest a correlation between immunological and hepatic effects and the ability to induce AHH activity and that the most toxic congeners are those that resemble the structural configuration of 2,3,7,8-TCDD. This relationship further supports the idea of a common receptor-mediated mechanism of action. Other PBB congeners (*ortho*-substituted) induce other types of effects, such as neurotoxicity, by yet unknown but Ah receptor independent mechanisms.

Some information on structure-promotion relationships for PBBs is available from studies that used two-stage liver and skin carcinogenesis models. In the liver promotion studies, development of enzyme-altered hepatic foci (putative preneoplastic lesions) was assessed in rats that were partially hepatectomized, initiated with diethylnitrosamine and promoted with PBBs (Buchmann et al. 1991; Dixon et al. 1988; Evans and Sleight 1989; Jensen and Sleight 1986; Jensen et al. 1982, 1983; Sleight 1985). Both MC-type (3,3',4,4',5,5'-hexabromobiphenyl and 3,3',4,4'-tetrabromobiphenyl) and PB-type (2,2',4,4',5,5'-hexabromobiphenyl) congeners showed hepatic promoting activity with varying potencies, indicating that this response is partially Ah-receptor independent. FireMaster BP-6 was a more effective promoter than its major constituent congener 2,2',4,4',5,5'-hexabromobiphenyl (Jensen et al. 1982), which also indicates that other congeners are very effective as promoters, or possibly that the combination of congeners with mixed- or PB-type activity have a synergistic or additive effect. Although both MC- and PB-type congeners promote two-stage hepatic tumor activity, it appears that the MC-type congeners may exert their effects indirectly by causing hepatotoxic (cytotoxic effects and necrosis), whereas the PB-type congeners may act as mitogens (stimulate cellular growth and division). In a skin tumor assay, HRS/J hairless mice were initiated with MNNG and promoted with PBBs (Poland et al. 1982). FireMaster FF-1 and 3,3',4,4',5,5'-hexabromobiphenyl were effective skin tumor promoters, but 2,2',4,4',5,5'-hexabromobiphenyl showed no activity, suggesting that, unlike rat liver tumor promotion, promoter activity in the mouse skin tumor model is Ah receptor-dependent. Another indication that promotion of tumors by PBBs is not solely an Ah-receptor mediated process is provided by the results of an *in vitro* gap junctional intercellular intercommunication assay (Kang et al. 1996). Gap junctional intercellular

intercommunication in normal human breast epithelial cells was inhibited by 2,2',4,4',5,5'-hexaCB in a dose-dependent manner, but not by the coplanar congener 3,3',4,4',5,5'-hexaCB. Inhibition of gap junctional intercellular communication is generally regarded as a mechanistic marker for tumor promotion (as well as several other toxicological end points).

Expression of the toxic response, which is species and strain dependent, is initiated by the binding of individual congeners with the Ah receptor. The responsiveness of a particular organ or cell depends on the affinity of the receptor for the ligand molecule (Goldstein and Safe 1989). For example, certain inbred strains of mice, typified by C57BL/6J, have a cytosolic Ah receptor protein with a relatively high binding affinity for inducers of benzo[a]pyrene hydroxylase such as 3-MC, ß-naphthoflavone, 2,3,7,8-TCDD, and other isostereomers of 2,3,7,8-TCDD. In contrast, other inbred mouse strains, such as DBA/2J, have reduced levels of the Ah receptor. Responsiveness to aromatic hydrocarbons is inherited in a simple autosomal dominant mode. Nonresponsiveness has been attributed to a mutation resulting in a receptor with a diminished affinity (Okey et al. 1989). This defective receptor is almost completely unresponsive to weak inducers such as 3-MC and has reduced sensitivity to more potent inducers such as 2,3,7,8-TCDD. Studies with PBBs have shown that treatment of C57BL/6J and DBA/2J with FireMaster BP-6 resulted in the induction of hepatic microsomal benzo[a]pyrene hydroxylase only in the C57BL/6J strain and aminopyrine N-demethylase (PB inducible) in both strains of mice (Robertson et al. 1984a). However, 3,3',4,4'-tetrabromobiphenyl, a more potent MC-type inducer than the BP-mixture, induced benzo[a]pyrene hydroxylase in both strains of mice but did not induce aminopyrine N-demethylase in either strain of mice. Also, after treatment with the dioxin-like congener 3,3',4,4'-tetrabromobiphenyl, thymic atrophy was only observed in the responsive strain (Robertson et al. 1984a). In general, studies summarized in Section 3.2 in which more than one strain was tested (mice or other species) do not address the possible strain-dependency of the toxic responses observed. It must be mentioned, however, that differences in the response between tissues, strains, or species, do not exclusively indicate differences in receptor affinities, but most likely reflect the fact that the battery of enzyme activities (see below) controlled by the Ah locus varies within the tissue, strain, and animal species.

Initial binding of a PBB congener to the Ah receptor is followed by an activation or transcription step and subsequent accumulation of occupied nuclear receptor complexes. These complexes interact with a specific DNA sequence in the CYP1A1 gene (which regulates the expression of cytochrome P-450IA1 isozymes), changing its secondary or supersecondary structure (Elferinck and Whitlock 1990), which leads to enhancement of the CYP1A1 gene expression. A specific nucleotide sequence present in multiple copies to which the nuclear complex binds has been identified (Denison et al. 1989). Ultimately,

newly synthesized enzymes and macromolecules resulting from the pleiotropic response to the PBB-receptor complex are believed to be responsible for many of the effects caused by PBBs and other halogenated aromatic hydrocarbons (see Chapter 2).

No studies were located regarding the mechanism of endocrine effects (thyroid toxicity, estrogenicity) of PBBs.

Polybrominated Diphenyl Ethers. Some PBDEs have been shown to bind to the Ah receptor in a limited number of studies. For example, Meerts et al. (1998) indirectly examined the Ah receptor-mediated properties of 17 PBDE congeners in a recombinant H4II rat hepatoma cell line showing Ah receptor mediated expression of a luciferase reporter gene. The tested congeners varied from dibromo-substituted to heptabromo-substituted, and with the exception of 4,4'-diBDE and 3,3',4,4'-tetraBDE, all had at least one *ortho* substitution. Seven of the congeners showed luciferase expression, indicating their ability to activate the Ah receptor. The only discernable pattern of receptor activation that appeared to emerge from these results was that greater receptor activation was obtained with the penta- and hexaBDEs than with tri- and tetraBDEs. More recently, Chen et al. (2001) studied the affinities of a series of 18 PBDE congeners and 3 commercial PBDE mixtures for rat hepatic Ah receptor through competitive binding assays. The analysis showed that both the congeners and mixtures had binding affinities 10^{-2}–10^{-5} times that of 2,3,7,8-TCDD. The congener with the highest affinity among the tested congeners was 2,2',3,4,4'-pentaBDE, although its relative binding affinity was only 2% that of 2,3,7,8-TCDD. No binding activity could be determined for a decaBDE mixture. In contrast with PCBs, the binding affinities did not appear to relate to the planarity of the molecule, which according to Chen et al. (2001), was possibly due to the fact that the large size of bromine atoms expands the Ah's receptor binding site.

The enzyme induction properties of PBDEs have been less studied than for other structurally similar chemicals, but the existing information suggests that they can be classified as mixed-type inducers of hepatic microsomal monooxygenases (Darnerud et al. 2001; de Wit 2002; Hardy 2002b). Few studies have examined the structure-induction relationships for PBDEs. Chen et al. (2001) examined the ability of 12 PBDE congeners and 3 commercial mixtures to induce EROD activity in chick and rat hepatocytes, in liver cell lines from rainbow trout, rat, and human, and in a human intestinal cell line. The number of bromine substitutions in the congeners tested ranged from 3 to 7. In all cell types, 3,3',4,4'-tetraBDE, 2,2',4,4',6-pentaBDE, 2,3',4,4'-tetraBDE, and 3,3',4,4',5-pentaBDE were the strongest inducers. Congeners 2,2',4,4',5,5'-hexaBDE and 2,2',3,4,4',5',6-heptaBDE were weak inducers in all cell types, whereas 2,3',4,4'-tetraBDE and 2,2', 3,4,4'-pentaBDE were very weak inducers in rat hepatocytes and

inactive in the other cells. Congeners 2,2',4,4'-tetraBDE and 2,2'4,4',5-pentaBDE, which are prominent in the environment, were not inducers in any cell line, and neither were 2,4,4'-triBDE, 2,2',4,4',5,6'-hexa-BDE, or the penta-, octa-, or decaBDE mixtures. For those congeners that had measurable EROD induction activity, their relative potencies were 10^{-3}–10^{-6} that of 2,3,7,8-TCDD. In general, the EROD induction activity paralleled the strength of the Ah receptor binding with the notable exception of 2,2',3,4,4'-pentaBDE, which despite its relatively strong Ah receptor binding affinity (see above), showed no evidence of activating the Ah receptor to its dioxin-response element (DRE) binding form and was only a weak EROD inducer.

Studies on structure-toxicity relationships have not been conducted, but relevant information is available for PCDEs. Howie et al. (1990) examined the immunotoxic potencies of various PCDE congeners on the inhibition of the splenic PFC response to SRBC antigen and found the following potency order: 2,3,3',4,4',5-hexaCDE > 3,3',4,4',5-pentaCDE > 2, 3',4,4',5-pentaCDE > 3,3',4,4'-tetraCDE > 2,2',4,4',5,5'-hexaCDE > 2,2',4,5,5'-pentaCDE > 2,2',4,4',5,6'-hexaCDE. In general, this potency order paralleled their potencies as inducers of hepatic microsomal AHH and EROD. Worth noticing is the fact that the resulting ranking order of potency did not follow the order that would have been expected for a response known to be Ah receptor-mediated, such as the inhibition of the PFC response to challenge with SRBC antigen. For example, the coplanars 3,3',4,4'-tetraCDE and 3,3',4,4',5-pentaCDE were less immunotoxic than their respective monoortho-substituted analogs; this was true also for their enzyme induction potencies. These findings showed that increasing *ortho*-substitution is less effective in reducing the "dioxin-like" activity of these compounds. Howie et al. (1990) suggested that the ether bridge in the polyCDE molecules increases the bond length between the two phenyl rings, thus diminishing the effects of *ortho* substituents on the biochemical and toxic potencies of these compounds.

Evidence for thyroid hormone involvement in PBDE toxicity includes observations in rats and mice that were orally exposed to commercial mixtures of deca-, octa-, or pentaBDE (see Section 3.2.2.2, Endocrine Effects). The main findings include (1) histological changes in the thyroid indicative of glandular stimulation (e.g., follicular cell hyperplasia similar to that induced by a hypothyroid state) (IRDC 1976; Norris et al. 1973, 1975b; NTP 1986; WIL Research Laboratories 1984), and (2) decreased serum thyroxine (T_4) levels with no changes in serum TSH (Darnerud and Sinjari 1996; Fowles et al. 1994; Hallgren and Darnerud 1998;WIL Research Laboratories 1984; Zhou et al. 2001, 2002). Considering these data, the structural resemblance of PBDEs to T_4, and information from studies of individual congeners as summarized below, it appears that, depending on dose, duration, and mixture/congener, PBDEs can disrupt the production, transport, and disposition of thyroid hormones.

The mechanism(s) by which PBDEs decrease serum levels of T_4 is not completely characterized, but appears to involve induction of hepatic microsomal enzymes, which could lead to increased metabolic clearance of thyroid hormones and increased binding affinity to thyroid hormone receptors and transport proteins. The apparent lack of effect of PBDEs on TSH levels suggests that direct effects on the thyroid leading to inhibition of T_4 synthesis are unlikely. The induction of hepatic UDPGT by PBDEs has been demonstrated in several studies (Fowles et al. 1994; Hallgren et al. 2001; Zhou et al. 2001, 2002) and this could increase the UDPGT-catalyzed deactivation and excretion of T_4 (i.e., the conjugation of T_4 with glucuronic acid). An indication that increased UDPGT activity may not be the main mechanism for the reduced T_4 levels is provided by Hallgren et al. (2001), who found that exposure to $18 mg/kg/day pentaBDE for 14 days caused serum T_4 reductions in both mice and rats with no effect on UDPGT activity in the mice and increased UDPGT in the rats only at higher dose levels. In contrast, the decreases in serum T_4 correlated with the induction of microsomal phase I enzymes (EROD and MROD). As discussed below, increased microsomal enzyme activity could increase the formation of hydroxylated PBDE metabolites that can bind to T_4 plasma transport proteins. This would serve to increase the number of occupied sites on T_4-binding proteins and subsequently result in decreased serum levels of T_4.

The possible interaction of PBDEs with T_4 binding to human transthyretin (TTR) was investigated in an *in vitro* competitive binding assay (Meerts et al. 1998, 2000). Testing of 17 congeners, ranging from di- to heptaBDEs, showed that none of the parent compounds competed with T_4 for binding to human TTR. Incubation of the congeners with rat liver microsomes induced by PB (CYP2B enriched), β-naptho-flavone (CYPIA enriched), or clofibrate (CYP4A3 enriched) indicated that metabolism is necessary to compete with T_4-TTR binding and that potency is likely to be both congener and metabolic enzyme-specific. The CYP2B-enriched liver microsomes were the most potent, causing 9 of the 17 congeners to generate metabolites (not identified) that were effective in displacing T_4 from TTR (60% inhibition): 4,4'-diBDE, 2,4,4'-triBDE, 2,4,6-triBDE, 2,2',4,4-tetraBDE, 2,2',4,6'-tetraBDE, 2,4,4',6-tetraBDE, 3,3,4,4'-tetraBDE, 2,2',4,4',6-pentaBDE, and 2,3',4,4',6-pentaBDE. No T_4-TTR inhibition occurred with the higher brominated diphenyl ethers (i.e., 2,2',3,4,4',5'-hexaBDE, 2,2',4,4',5,5'-hexaBDE, 2,3,4,4',5,6-hexaBDE, and 2,3,3',4,4',5,6-heptaBDE), although it was not verified that these PBDEs were metabolized during the *in vitro* microsomal incubations. Three pure hydroxylated PBDEs, synthesized for their structural resemblance with the thyroid hormones 3,5-diiodothyronine (3,5-T_2), 3,3',5-triiodo-thyronine (T_3), and 3,3',5,5'-tetraiodothyronine (T_4), were also tested in the T_4-TTR competition binding assay. The relative potencies showed that the T_4-like (2,6-dibromo-4-[2,4,6-tribromophenoxy]phenol) and T_3-like (2-bromo-4-[2,4,6-tribromophenoxy]phenol) hydroxylated PBDEs were 1.42- and 1.22-fold more potent, respectively, than T_4, and the percentage competition at 500 nM exceeded that of the natural

ligand; the T_2-like hydroxylated PBDE (4-[2,4,6-tribromophenoxy]phenol) showed low affinity for TTR (0.41-fold less potent than T_4). Because the PBDEs were able to compete with T_4-TTR binding only after metabolic conversion by induced rat liver microsomes, and considering that thyroid hormones are also hydroxy-halogenated diphenyl ethers, the results of this study suggest an important role for hydroxylation in the mechanism of thyroid toxicity.

4'-Hydroxyl derivatives of 1,3,5-triBDE, 1,3,3',5'-tetraBDE, and 1,3,3',5,5'-pentaBDE were tested for affinity to the human thyroid hormone receptor proteins THR-α and THR-β *in vitro* (Marsh et al. 1998). These congeners were tested because they theoretically show the highest structural similarity to T_4 and T_3. None of the hydroxylated derivatives effectively competed with the thyroid hormones for binding to either receptor (affinities were 4–>1,000 times less than for T_4 and T_3). Because the tested congeners were the most likely to have affinity for the thyroid hormone receptor, it is likely that other hydroxylated PBDE congeners will have even lower potential for receptor binding.

2,2',4,4'-TetraBDE did not competitively inhibit binding of T_4 to sites in rat choroid plexus homogenates following *in vitro* or *in vivo* exposure (Sinjari et al. 1998). In the *in vivo* study, choroid plexus homogenate from rats that were orally treated with 6 or 18 mg/kg/day for 14 days showed T_4 binding that was 80 and 63%, respectively, of that in controls. TTR is the only T_4-binding protein synthesized by the choroid plexus and is the main protein involved in T_4 transport from blood to brain in both rats and humans (Sinjari et al. 1998).

The estrogenic and antiestrogenic activities of several PBDE congeners and three hydroxylated PBDEs were tested *in vitro* using human breast cell line assays based on estrogen receptor (ER)-dependent luciferase reporter gene expression (Meerts et al. 2001). The hydroxylated PBDEs, tribromophenoxy)phenol, 2-bromo-4-(2,4,6-tribromophenoxy)phenol, and 2,6-dibromo-4-(2,4,6-tri-bromophenoxy)phenol, have bromine substitution patterns similar to those of the thyroid hormones T_2, T_3, and T_4, respectively. Eleven of 17 PBDE congeners showed estrogenic activity (dose-dependent luciferase induction) in the ER-CALUX assay with T47D.Luc cells, although the most potent PBDE congeners (2,2',4,4',6-pentaBDE > 2,4,4',6-tetraBDE > 2,2',4,6'-tetraBDE > 2,4,6-triBDE > 2,3',4,4',6-pentaBDE) had EC_{50} values that were 250,000–390,000 times less potent than 17β-estradiol (E_2). In contrast, the T_3- and T_2-like hydroxylated PBDEs showed estrogenic potencies exceeding that of E_2 (no estrogenic activity was induced by the T_4-like hydroxylated PBDE). Antiestrogenic potencies were determined in the ER-CALUX assay by treating T47D.Luc cells with the PBDEs and hydroxylated PBDEs in the presence of E_2. Only 2,2',4,4',5,5'-hexaBDE, 2,3,4,4',5,6-hexaBDE, and

2,3,3',4,4',5,6-heptaBDE, which did not induce luciferase activity alone, caused reductions in E_2-induced luciferase activity. Three of the compounds with potent estrogenic activity in the ER-CALUX assay, 2,4,6-triBDE, 2,2',4,4',6-pentaBDE, and 4-(2,4,6-tribromophenoxy)phenol, were also tested for estrogenicity in ERα-specific and ERβ-specific human embryonic kidney cell lines (293-ERα-Luc and ERβs-Luc cells, respectively). The hydroxylated PBDE was potent in the ERα-specific cells (maximum luciferase induction similar to E_2) and also showed activity in the ERβ-specific cells (maximum 50% induction compared to E_2), whereas the ERα- and ERβ-specific cell lines were less responsive to 2,4,6-triBDE (34.2 and 7.8% induction compared to E_2) and 2,2',4,4',6-pentaBDE (. 20 and < 2% relative induction). These results indicate that pure and hydroxylated congeners of PBDEs can be agonists of both ERα and ERβ receptors and that metabolism of PBDEs may produce more potent pseudoestrogens. The common structural features among the estrogenic PBDEs in this study are two *ortho* (2,6)-bromine atoms on one phenyl ring, at least one *para*-bromine atom (preferably on the same phenyl ring as the *ortho* bromines), and nonbrominated *ortho*-*meta* or *meta* carbons on the other phenyl ring (Meerts et al. 2001).

3.5.3 Animal-to-Human Extrapolations

Residue levels of PBBs and PBDEs in humans reflect multiple exposure pathways and congener-specific elimination and thus, in general, represent steady state body burdens that do not match the congener profiles in the original exposure sources. For example, profiles of PBB and PBDE congeners in human milk do not resemble the pattern of any of the commercial mixtures, as illustrated by the finding that the major PBDE congener in milk from Swedish mothers was 2,2',4,4'-tetraBDE (BDE-47), which comprised approximately 55% of the total PBDEs (Darnerud et al. 1998). As discussed in Chapter 6, residue analyses indicate that tetra- to hexa-congeners predominate in humans, aquatic mammals, birds, fish, and other biota, indicating that the biological fate of PBB and PBDE congeners is qualitatively similar in various animal species. Interspecies differences in the quantitative disposition of PBBs and PBDEs may exist. For example, the metabolism and urinary excretion of a single oral dose of 2,2',4,4'-tetraBDE was significantly slower in rats than in mice (Orn and Klasson-Wehler 1998).

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Colborn and Clement (1992), was also used in 1996 when Congress mandated the Environmental Protection Agency (EPA) to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), which in 1998 completed its deliberations and made recommendations to EPA concerning *endocrine disruptors.* In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous

estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral functions. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

Concern has been raised that many industrial chemicals, including PBBs and PDBEs, are endocrine-active compounds capable of having widespread effects on humans and wildlife (Colborn et al. 1993; Crisp et al. 1998; Daston et al. 1997; Safe and Zacharewski 1997). Particular attention has been paid to the possibility of these compounds mimicking or antagonizing the action of estrogen. Estrogen influences the growth, differentiation, and functioning of many target tissues, including female and male reproductive systems, such as mammary gland, uterus, vagina, ovary, testes, epididymis, and prostate. In addition, there is evidence that some of these environmentally-persistent chemicals alter the thyroid hormone system, which is a very important system for normal structural and functional development of sexual organs and the brain.

Polybrominated Biphenyls. PBBs have the potential to interact with the endocrine system based on effects that mainly include changes in levels of thyroid and female reproductive hormones. No studies were located that investigated the estrogenic and antiestrogenic activity of PBBs *in vitro* or *in vivo* at the level of the estrogen receptor.

The thyroid gland is an unequivocal target of PBBs in animals, and evidence in humans is suggestive of a similar relationship. A spectrum of effects has been observed in rats exposed for acute and intermediate durations, ranging from decreases in serum levels of T_4 and T_3 to histological and ultrastructural changes in the follicles (Akoso et al. 1982b; Allen-Rowlands et al. 1981; Byrne et al. 1987; Gupta and Moore 1979; Kasza et al. 1978a; Norris et al. 1975a, 1975b; NTP 1983; Sepkovic and Byrne 1984; Sleight et al. 1978). The preponderance of these studies tested FireMaster FF-1 or FireMaster BP-6 in rats, although chronic exposure to FireMaster FF-1 induced thyroid follicular hyperplasia in mice (NTP 1992). Thyroid effects also occurred in offspring of treated rats and pigs (Meserve et al. 1992; Werner and Sleight 1981). Effects in workers exposed to unspecified PBBs and/or decabromobiphenyl included increased serum

FSH, low or borderline low serum T_4, and increased thyroid antimicrosomal antibody titers (Bahn et al. 1980).

Serum levels of the adrenal hormones corticosterone B, dehydroepiandrosterone, and dehydro-epiandrosterone sulfate were decreased in rats fed $0.25 mg/kg/day FireMaster BP-6 for 5–7 months (Byrne et al. 1988). Serum corticosterone levels and adrenal weight did not change in rats exposed to #6 mg/kg/day of an unspecified PBB mixture for a shorter duration of 20 days (Castracane et al. 1982).

Increased menstrual cycle duration and prolonged implantation bleeding were observed in female monkeys fed FireMaster FF-1 in approximate daily dose levels of 0.012 mg/kg for 7 months before breeding and during pregnancy (Allen et al. 1979; Lambrecht et al. 1978). A corresponding decrease in serum levels of progesterone suggests that the reproductive effects in the monkeys are related to PBB-induced endocrine imbalance. Implantation was completely blocked in 40–67% of female rats treated with FireMaster BP-6 by gavage in dose levels $28.6 mg/kg on alternate days between gestation days 0 and 14 (Beaudoin 1979).

Delayed vaginal opening, an effect suggesting retarded sexual maturation, was observed in F_1 generation rats whose only PBB exposure was from the mothers fed a diet providing 5 mg/kg/day FireMaster FF-1 from day 8 of pregnancy until weaning at 28 days postpartum (McCormack et al. 1981).

Two studies of women exposed during the Michigan contamination episode found no associations between serum levels of PBBs and breast feeding (Michels Blanck et al. 2000b; Thomas et al. 2001). Determinants of PBB serum decay were investigated in women who had a mean initial PBB level of 20.9 ppb, a mean time between the first and last measurement of 4.2 years, and at least two measurements taken when they were not pregnant (Michels Blanck et al. 2000b). The median PBB half-life was estimated to be 13.5 years. Subject-specific decay rates were regressed on various predictor variables. Results included the finding that breast feeding, as either a continuous variable or as categorized by duration (<3, 3–9, or >9 months), was not associated with serum PBB decay, although increasing number of pregnancies was weakly associated with a slower rate of serum PBB decay (the effect had borderline statistical significance). Additional information on the design and results of this study is provided in Section 3.8.1. Thomas et al. (2001) found no relationship between serum levels of PBBs and the frequency and duration of lactation in Michigan women. Characteristics of the study cohort included a mean initial serum PBB level of 17.5 ppb, an estimated mean serum PBB level at delivery of 9.4 ppb, mean duration of breast-feeding as main source of nutrition of 2.6 months, and mean total duration of

breast feeding of 4.1 months. Exposure was treated as a categorical variable by dividing the women into groups of low (reference) exposure (#1 ppb), moderate exposure (>1–#7 ppb), and high exposure (>7 ppb), and three outcomes of interest were analyzed: (1) the decision to breast feed (yes/no), (2) the duration (months) of breast-feeding as the main source of nutrition, and (3) the total duration (months) of breast-feeding. None of the three outcomes was significantly associated with serum PBB levels. Additional information on the design and results of this study is provided in Section 3.2.2.5.

The issue of breast cancer has received attention following reports of high levels of organochlorine compounds in breast cancer patients. A relationship between increasing serum levels of PBBs and increasing risk of breast cancer was indicated in case-control studies of women exposed during the Michigan contamination episode (Henderson et al. 1995; Hoque et al. 1998), but the results are only suggestive due to factors such as small number of cases, insufficient information on known breast cancer risk factors, and confounding exposures to other organochlorine chemicals. The evidence for an association between breast cancer and PCBs is also not conclusive (Agency for Toxic Substances and Disease Registry 2000), and the hypothesis that environmental exposure to PCBs can cause breast cancer in humans is controversial (Safe and Zacharewski 1997; Wolff and Toniolo 1995). Overall, the evidence for an association between breast cancer and PBBs is inconclusive and needs further study.

Polybrominated Diphenyl Ethers. Results of *in vitro* estrogen receptor and thyroid hormone transport protein binding assays and *in vivo* studies of thyroid hormone homeostasis indicate that PBDEs have the potential to disrupt normal endocrine function.

The estrogenic and antiestrogenic activities of 17 PBDE congeners and 3 hydroxylated PBDEs were tested *in vitro* using human breast cell line assays based on ER-dependent luciferase reporter gene expression (Meerts et al. 2001). Eleven of 17 PBDE congeners showed estrogenic activity (dose-dependent luciferase induction) in the ER-CALUX assay with T47D.Luc cells, although the most potent PBDE congeners (2,2',4,4',6-pentaBDE > 2,4,4',6-tetraBDE > 2,2',4,6'-tetraBDE > 2,4,6-triBDE > 2,3',4,4',6-pentaBDE) had EC_{50} values that were 250,000–390,000 times less potent than that of the natural ligand, 17β-estradiol (E_2). In contrast, two of the hydroxylated PBDEs, 4-(2,4,6-tribromophenoxy)phenol, 2-bromo-4-(2,4,6-tribromophenoxy)phenol (which have bromine substitution patterns similar to the thyroid hormones T_2 and T_3, respectively) had estrogenic potencies exceeding that of E_2. Three of the compounds with potent estrogenic activity in the ER-CALUX assay, 2,4,6-triBDE, 2,2',4,4',6-pentaBDE, and 4-(2,4,6-tribromophenoxy)phenol, were also tested for estrogenicity in ERα-specific and ERβ-specific human embryonic kidney cell lines (293-ERα-Luc and

ERβs-Luc cells, respectively). The hydroxylated PBDE was potent in the ERα-specific cells (maximum luciferase induction similar to E_2) and also showed activity in the ERβ-specific cells (maximum 50% induction compared to E_2), whereas the ERα- and ERβ-specific cell lines were less responsive to 2,4,6-triBDE and 2,2',4,4',6-pentaBDE. These results indicate that pure congeners of PBDEs can be agonists of both ERα and ERβ receptors and that metabolism to hydroxylated PBDEs may increase estrogenic potency.

The same 17 PBDE congeners and three hydroxylated PBDEs were also tested for possible interaction with T_4 binding to human TTR, a plasma transport protein of thyroid hormones, in an *in vitro* competitive binding assay (Meerts et al. 1998, 2000). None of the pure congeners competed with T_4 for binding to human TTR. Incubation of the congeners with rat liver microsomes induced by PB (CYP2B enriched), β-napthoflavone (CYPIA enriched), or clofibrate (CYP4A3 enriched) indicated that 9 of the 17 pure congeners generated metabolites (not identified) that were able to displace T_4 from TTR: 4,4'-diBDE, 2,4,4'-triBDE, 2,4,6-triBDE, 2,2',4,4-tetraBDE, 2,2',4,6'-tetraBDE, 2,4,4',6-tetraBDE, 3,3,4,4'-tetraBDE, 2,2',4,4',6-pentaBDE, and 2,3',4,4',6-pentaBDE. Testing of the three known hydroxylated PBDEs, used for their structural resemblance with the thyroid hormones 3,5-diiodothyronine (3,5-T_2), 3,3',5-triiodothyronine (T_3), and 3,3',5,5'-tetraiodothyronine (T_4) showed that the T_4-like (2,6-dibromo-4-[2,4,6-tribromophenoxy]phenol) and T_3-like (2-bromo-4-[2,4,6-tribromophenoxy]phenol) hydroxylated PBDEs were 1.42- and 1.22-fold more potent, respectively, than T4; the T_2-like hydroxylated PBDE (4-[2,4,6-tribromophenoxy]phenol) showed low affinity for TTR (0.41-fold less potent than T_4). Because the PBDEs were able to compete with T_4-TTR binding only after metabolic conversion by induced rat liver microsomes, and considering that thyroid hormones are also hydroxy-halogenated diphenyl ethers, the results of this study suggest an important role for hydroxylation in the mechanism of thyroid toxicity.

2,2',4,4'-TetraBDE did not competitively inhibit binding of T_4 to sites in rat choroid plexus homogenates following *in vitro* or *in vivo* exposure (Sinjari et al. 1998). TTR is the only T_4-binding protein synthesized by the choroid plexus and is the main protein involved in T_4 transport from blood to brain in both rats and humans (Sinjari et al. 1998).

4'-Hydroxyl derivatives of 1,3,5-triBDE, 1,3,3',5'-tetraBDE, and 1,3,3',5,5'-pentaBDE were tested for affinity to the human thyroid hormone receptor proteins, THR-α and THR-β, *in vitro* (Marsh et al. 1998). These congeners theoretically show the highest structural similarity to T_4 and T_3. None of the hydroxylated derivatives effectively competed with the thyroid hormones for binding to either receptor (affinities were 41–>1,000 times less than for T_4 and T_3). Because the tested congeners were the most

likely to have affinity for the thyroid hormone receptor, it is likely that other hydroxylated PBDE congeners will have even lower potential for receptor binding.

Studies of serum hormone levels and organ histology indicates that the thyroid is a particularly sensitive target of PBDEs. Reduced serum T_4 levels and follicular cell hyperplasia have been consistently observed in rats and mice orally exposed to PBDEs. Acute-duration studies showed decreases in serum T_4 in rats exposed to $10 mg/kg/day octaBDE or $30 mg/kg/day pentaBDE for 4 days and in rats and mice exposed to $18 mg/kg/day pentaBDE for 14 days (Darnerud and Sinjari 1996; Hallgren et al. 2001; Zhou et al. 2001). Effects observed in intermediate-duration studies include thyroid hyperplasia in rats exposed to $8 mg/kg/day octaBDE for 30 days (Norris et al. 1973, 1975a, 1975b) and reduced serum T_4 in rats exposed to $10 mg/kg/day pentaBDE for 90 days (WIL Research Laboratories 1984). Exposure to pentaBDE on gestation day 6 through postnatal day 21 caused serum T_4 reductions at 30 mg/kg/day in maternal rats and at $10 mg/kg/day in their offspring (Zhou et al. 2002). Intermediate-duration exposure to a 77% decaBDE/22% nonaBDE commercial mixture caused thyroid hyperplasia in rats at doses of $80 mg/kg/day for 30 days (Norris et al. 1973, 1975a, 1975b). Chronic (103-week) exposure to high-purity decaBDE ($97%) did not induce thyroid histopathological changes in rats at #2,550 mg/kg/day, although follicular cell hyperplasia developed in mice exposed to 2,240 mg/kg/day (NTP 1986).

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed. In general, methodology has not yet been developed for assessing health risks of chemicals in children.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6 Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age

(Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

Children are exposed to PBBs and PBDEs in the same manner as the general population, primarily via consumption of contaminated foods. Exposure also may occur by transfer of PBBs and PBDEs that have accumulated in women's bodies to the fetus across the placenta. Because PBBs and PBDEs are lipophilic substances, they can additionally accumulate in breast milk and be transferred to nursing infants.

Placental transfer, although it may be limited in absolute amounts, is a concern because of possible effects of PBBs and PBDEs on sensitive immature tissues, organs, and systems, with potentially serious long-lasting consequences. Transfer of PBBs and PBDEs via breast milk could be relatively considerable and, like prenatal exposure, has the potential to contribute to altered development.

Although embryos, fetuses, and nursing infants may be exposed to relatively high amounts of PBBs and PBDEs during sensitive periods of development, it is not known if the susceptibility of children to the health effects of these chemicals differs from that of adults. Younger children may be particularly vulnerable to PBBs and PBDEs because, compared to adults, they are growing more rapidly and are generally expected to have lower and distinct profiles of biotransformation enzymes, as well as much smaller fat depots for sequestering these lipophilic chemicals. No specific information was located regarding the pharmacokinetics of PBBs in children or nutritional factors that may influence the absorption of PBBs.

No biomarkers of exposure or effect for PBBs or PBDEs have been validated in children or in adults exposed as children. There also are no biomarkers in adults that identify previous childhood exposure. No studies were located regarding interactions of PBBs or PBDEs with other chemicals in children or adults. No information was located regarding pediatric-specific methods for reducing peak absorption following exposure to PBBs or PBDEs, reducing body burden, or interfering with the mechanism of action for toxic effects. In addition, no data were located regarding whether methods for reducing toxic effects in adults might be contraindicated in children.

Polybrominated Biphenyls. Information on health effects of PBBs in children is available from several studies of the Michigan contamination episode. A 1976 medical history questionnaire study of 342 Michigan children likely to have been exposed to PBBs found that the number of subjectively reported symptoms of ill health, including several symptoms of neurological effects, did not increase with increasing serum PBB levels (assayed in 1976), but rather decreased; general neurological examinations did not reveal a pattern of abnormality among the Michigan children (Barr 1980). Studies of fetal mortality rates in Michigan (Humble and Speizer 1984) and of physical and neuropsychological development in Michigan children exposed during the contamination episode (Schwartz and Rae 1983; Seagull 1983; Weil et al. 1981) did not conclusively correlate the ingestion of PBBs with effects, as summarized below.

Examination of approximately 100 children presumably exposed *in utero* or in early infancy during the peak of the Michigan contamination episode and whose families lived on farms known to be contaminated with PBBs has not revealed any consistent or marked abnormalities. No significant abnormalities were found by physical and neuropsychological examination of 33 of these exposed children when they had a mean age of 37.2 months, compared with a group of 20 age-matched, non-exposed control children (Weil et al. 1981). However, subjective interviews with parents suggested that more exposed children than control children had frequent upper respiratory illnesses such as colds, runny noses, and sore throats (Weil et al. 1981). Administration of 5 of 18 possible neuropsychological development tests from the McCarthy Scales of Children's Abilities to 19 of these exposed children at .2.5–4 years of age showed a statistically significant negative correlation between PBB levels in fat tissue and developmental abilities in four of the five tests (Seagull 1983). Subsequent administration of the full battery of 18 neuropsychological tests, as well as I.Q. tests, to the same group of children when .4–6 years old, found that the exposed children's performances were within the normal range in all areas assessed (Schwartz and Rae 1983). Due mainly to the small data set and the inconsistency of the results, the available data do not conclusively establish or eliminate the possibility that *in utero* and early infancy exposure to PBBs might adversely affect the development of human children.

Neurobehavioral alterations have been observed in animals following gestational and lactational exposure to PBBs. Performance deficits in tests of operant behavior were seen in 6-month-old offspring of rats that were exposed to $0.2 mg/kg/day of FireMaster BP-6 by gavage from day 6 of gestation until weaning (Henck and Rech 1986), but not in 75-day-old offspring of rats exposed to $0.5 mg/kg/day for 4 weeks prior to mating (Geller et al. 1985). Effects on acquisition of forward locomotion, cliff avoidance, cage emergence, and open-field activity were found in offspring of rats exposed to $0.2 mg/kg/day of FireMaster BP-6 in the diet from day 6 of gestation through Pnd 24 and tested through Pnd 60 (Henck et al. 1994). Testing of mouse offspring at 30–120 days of age following maternal exposure to FireMaster FF-1 by gavage on every other day during gestation and through weaning showed altered negative geotaxis and avoidance response latencies at $3 mg/kg/day and reduced acoustic startle responsiveness and motor activity at 10 mg/kg/day (Tilson 1992).

Animal studies of FireMaster FF-1 and FireMaster BP-6 have also shown that hexabromobiphenyl PBB mixtures can induce non-neurological developmental toxicity. Embryolethal effects or increased mortality among nursing young were observed in rats (Beaudoin 1977, 1979; Groce and Kimbrough 1984) and mice (Luster et al. 1980) after oral exposure during gestation and in monkeys after exposure before conception and during pregnancy (Allen et al. 1979; Lambrecht et al. 1978). Structural

malformations in fetuses, including cleft palate, were also observed in rats (Beaudoin 1977) and mice (Corbett et al. 1975) after exposure to these PBBs during gestation. Increased incidences of fetuses with extra ribs were found in a study of rats orally exposed to a commercial octabromobiphenyl mixture during gestation (Waritz et al. 1977), although decabromobiphenyl was not embryotoxic, fetotoxic, or teratogenic in rats (Millischer et al. 1980). Other studies with FireMaster FF-1 and FireMaster BP-6 found that body weight gain was reduced in the offspring of rats and mice after exposure during gestation and/or lactation (Corbett et al. 1975; Groce and Kimbrough 1984; McCormack et al. 1981, 1982c; Meserve et al. 1992). Liver effects, including increased liver weight and hepatic cytochrome P-450 enzymic activity, hepatocyte enlargement, vacuolization, and/or other degenerative changes, occurred in the offspring of rats, mice, and swine fed FireMaster FF-1 or FireMaster BP-6 during gestation and/or lactation (Chhabra et al. 1993; Moore et al. 1978; NTP 1992; Werner and Sleight 1981).

Other effects in offspring of animals exposed to PBBs during gestation and lactation include altered thyroid hormone levels. Serum T_4 levels were reduced in 15-day-old offspring of rats that were exposed to 2.5 mg/kg/day FireMaster BP-6 in the diet from Gd 0 through Pnd 15 (Meserve et al. 1992). The pups had received pituitary stimulation by an injection of corticotropin-releasing factor or adrenal stimulation by an injection of adrenocorticotropic hormone. Serum concentrations of T_3 and T_4 were significantly reduced in 4-week-old nursing offspring of swine that were fed $1.25 mg/kg/day dietary doses of FireMaster BP-6 during the second half of gestation and throughout lactation (Werner and Sleight 1981). These effects in offspring are consistent with evidence that the thyroid gland is an unequivocal target of PBBs in adult animals. A spectrum of thyroid effects, ranging from decreases in serum T_4 and T_3 levels to histological and ultrastructural changes in the follicles, has been documented in adult rats orally exposed to PBBs (mainly FireMaster BP-6 and FF-1) for acute and intermediate durations (Akoso et al. 1982b; Allen-Rowlands et al. 1981; Byrne et al. 1987; Gupta and Moore 1979; Kasza et al. 1978a; Norris et al. 1975a, 1975b; NTP 1983; Sepkovic and Byrne 1984; Sleight et al. 1978). Additionally, there is suggestive limited evidence of thyroid effects in adult humans; effects in workers exposed to unspecified PBBs and/or decaBDE included increased serum FSH, low or borderline low serum T_4, and increased thyroid antimicrosomal antibody titers (Bahn et al. 1980).

No information was located on possible immunological effects of PBBs in children, and data in adult humans are limited and largely inconclusive. Altered lymphocyte transformation responses among populations exposed to PBBs during the Michigan contamination episode have been reported in some studies (Bekesi et al. 1978; Roboz et al. 1985), but other investigations were not able to confirm these findings (Landrigan et al. 1979; Silva et al. 1979; Stross et al. 1981). No correlation can be established

between altered immune parameters and serum PBB levels based on the available data. Exposure to PBBs caused altered immune responses in a variety of animal species, which suggests that humans may also be affected. Studies in animals, mostly intermediate-duration studies in rodents, showed that a variety of immunological parameters such as spleen and thymus weights (Gupta and Moore 1979; Gupta et al. 1981; NTP 1983), antibody production (Loose et al. 1981), and lymphoproliferative responses (Howard et al. 1980; Luster et al. 1978, 1980) can be affected by treatment with commercial PBB mixtures, although some of these effects were only seen at PBB levels that cause overt toxicity (Luster et al. 1978, 1980).

Levels of PBBs in breast milk have been measured in women exposed as a result of the Michigan contamination episode. The milk concentrations of PBBs in women from the lower peninsula of Michigan (exposed area) were generally higher than in breast milk of females from the upper peninsula (farthest from the sources) (Brilliant et al. 1978). PBB levels in breast milk of five women from exposed farms ranged from 0.21–92.7 ppm (Cordle et al. 1978; Humphrey and Hayner 1976). On a lipid basis, the ratio of PBBs in breast milk to maternal serum was 107–122 to 1 and in adipose tissue to breast milk was 1.1–1.5 to 1 in a cohort of Michigan residents (Eyster et al. 1983; Landrigan et al. 1979). No monitoring information was located on PBBs in breast milk for U.S. populations outside of Michigan.

Determinants of PBB serum decay were investigated in Michigan women who had a mean initial PBB level of 20.9 ppb, a mean time between the first and last measurement of 4.2 years, and at least two measurements taken when they were not pregnant (Michels Blanck et al. 2000b). Assuming that PBBs reached equilibrium in the body before substantial amounts were eliminated and before the first serum measurements were taken, the authors estimated the median PBB half-life to be 13.5 years. Subject-specific decay rates were regressed on various predictor variables. Results of the analysis included the finding that an increasing number of pregnancies between the first and last measurement was associated with a slower rate of serum PBB decay (the effect had borderline statistical significance). Breast feeding as either a continuous variable or as categorized by duration (<3, 3–9, or >9 months) was not associated with serum PBB decay. Additional information on the design and results of this study is provided in Section 3.8.1. Another study of women exposed to PBBs during the Michigan contamination episode similarly found no relationship between serum levels of PBBs and the frequency and duration of lactation (Thomas et al. 2001). Characteristics of the study cohort included a mean initial serum PBB level of 17.5 ppb, an estimated mean serum PBB level at delivery of 9.4 ppb, a mean duration of breast-feeding as the main source of nutrition for 2.6 months, and a mean total duration of breast-feeding of 4.1 months. Exposure was treated as a categorical variable by dividing the women into groups of low (reference)

exposure (#1 ppb), moderate exposure (>1–#7 ppb), and high exposure (>7 ppb). Three outcomes of interest were analyzed: (1) the decision to breast feed (yes/no), (2) the duration (months) of breast-feeding as the main source of nutrition, and (3) the total duration (months) of breast-feeding. None of the three outcomes was significantly associated with serum PBB levels, even after controlling for various confounding determinants, including histories of previous breast-feeding and thyroid disorders. Additional information on the design and results of this study is provided in Section 3.2.2.5.

Polybrominated Diphenyl Ethers. No information was located on health effects of PBDEs in newborn or older children. Thyroid and neurobehavioral alterations have been observed in animals following pre- and/or postnatal exposure to commercial PBDE mixtures and single PBDE congeners, indicating that these are effects of concern in exposed children.

Serum levels of thyroid hormones were reduced in offspring of rats exposed to PBDEs during gestation and lactation and in rats exposed as weanlings. Exposure to pentaBDE from Gd 6 through the end of lactation caused serum T_4 decreases in maternal rats (on Gd 20 and Pnd 22) at 30 mg/kg/day and in their offspring (on Pnds 4 and 14) at $10 mg/kg/day (Zhou et al. 2002). Assessment of weanling (28-day-old) rats that were orally exposed to PBDEs for 4 days and evaluated for thyroid hormone changes on the day after the last exposure showed that octaBDE caused significantly reduced serum T_4 and T_3 levels at $10 and $60 mg/kg/day, respectively (Zhou et al. 2001). Similar exposure to pentaBDE caused serum T_4 and T_3 decreases at higher dose levels of $30 and $100 mg/kg/day, respectively, whereas decaBDE had no effect on levels of any thyroid hormones (T_4, T_3, or FSH). The results of these studies are consistent with findings of reduced serum T_4 hormone levels and follicular cell hyperplasia in adult rats and mice confirming that the thyroid is a sensitive target of PBDE toxicity, especially octaBDE and pentaBDE at doses as low as 8–10 mg/kg/day (Darnerud and Sinjari 1996; Hallgren et al. 2001; Norris et al. 1973, 1975a, 1975b; WIL Research Laboratories 1984). Also, *in vitro* evidence shows that PBDEs can effectively compete with T_4 for binding to the human transport protein transthyretin (Meerts et al. 1998, 2000).

Altered thyroid hormone homeostasis can potentially cause neurodevelopmental effects because development of the central nervous system is dependent on thyroid hormones, but very little information is currently available on potential neurotoxic effects of PBDEs. Data are essentially limited to the results of three behavioral tests in animals showing some alterations in spontaneous locomotion behavior and learning and memory ability in mice that were tested at 2 months of age and as adults (4 months) following neonatal exposure (Pnds 3, 10, or 19) to single low oral doses of the congeners 2,2',4,4'-tetra-

BDE and 2,2',4,4',5-pentaBDE (Eriksson et al. 1998, 1999, 2001). One of the studies found effects in mice treated at either 3 or 10 days of age, but not at 19 days of age, suggesting that there is a critical window for the induction of behavioral disturbances (Eriksson et al. 1999). No studies were located that evaluated neurological effects of PBDEs in animals exposed as adults.

No information is available regarding the immunosuppressive potential of PBDEs in young animals, and data in adult animals are essentially limited to findings in acute-duration oral studies of relatively high doses of pentaBDE. The plaque-forming splenic cell antibody response to injected sheep red blood cells was significantly reduced in mice exposed to 72 mg/kg/day pentaBDE for 14 days (Fowles et al. 1994), and *in vitro* production of IgG immunoglobulin from mitogen-stimulated splenocytes was reduced in mice exposed to 36 mg/kg/day pentaBDE for 14 days (Darnerud and Thuvander 1998). Other 14-day studies in mice found no changes in natural killer cell activity at dosages #72 mg/kg/day (Fowles et al. 1994) or numbers of splenic and thymic lymphocyte subsets at dosages #36 mg/kg/day (Darnerud and Thuvander 1998), although 18 mg/kg/day of the single congener 2,2',4,4'-tetraBDE caused significantly reduced numbers of total lymphocytes and CD4+, CD8+, and CD45R+ subtypes in spleen (Darnerud and Thuvander 1998).

Information on the reproductive toxicity of PBDEs is limited to a one-generation study of decaBDE in rats that found no exposure-related functional effects (Dow Chemical Co. 1975; Norris et al. 1975a, 1975b). Developmental toxicity studies have shown no evidence of teratogenicity of deca-, octa-, and pentaBDE in rats and rabbits, although fetotoxic effects, including skeletal ossification variations at maternally toxic doses have been observed (Argus Research Laboratories 1985b; Breslin et al. 1989; Dow Chemical Co. 1975, 1985; Life Science Research Israel Ltd. 1987; Norris et al. 1975a, 1975b; WIL Research Laboratories 1986).

Some PBDE congeners have estrogenic activity *in vitro* (Meerts et al. 2001). Although 11 of 17 PBDE congeners were active (ER-dependent luciferase induction) in the ER-CALUX assay using a human breast cell line, the most potent PBDE congeners (2,2',4,4',6-pentaBDE > 2,4,4',6-tetraBDE > 2,2',4,6'-tetraBDE > 2,4,6-triBDE > 2,3',4,4',6-pentaBDE) had EC_{50} values that were much less potent (250,000–390,000 times) than that of the natural ligand, 17β-estradiol (E_2). In contrast, two hydroxylated PBDEs, 4-(2,4,6-tribromophenoxy)phenol, and 2-bromo-4-(2,4,6-tribromophenoxy)phenol (which have bromine substitution patterns similar to the thyroid hormones T_2 and T_3, respectively) had estrogenic potencies exceeding that of E_2. Three of the congeners with potent estrogenic activity in the ER-CALUX assay, 2,4,6-triBDE, 2,2',4,4',6-pentaBDE, and 4-(2,4,6-tribromophenoxy)phenol, were also tested for

estrogenic activity in ERα-specific and ERβ-specific human embryonic kidney cell lines. The hydroxylated PBDE was potent in the ERα-specific cells (maximum luciferase induction similar to E_2) and showed some activity in the ERβ-specific cells (maximum 50% induction compared to E_2), whereas the ERα- and ERβ-specific cell lines were less responsive to 2,4,6-triBDE and 2,2',4,4',6-pentaBDE. These *in vitro* findings suggest that pure congeners are weak ER agonists compared to E_2 and that hydroxylated metabolites are more likely to be responsible for physiological responses to PBDEs as indicated by the ER-dependent luciferase induction assay.

PBDEs are pervasive environmental contaminants that bioaccumulate in the mother's body and can be transferred to infants through the placenta and breast milk. Considering the continued widespread production and use of PBDEs, particularly commercial pentaBDE mixtures whose constituent tetra- to hexaBDE congeners are highly bioaccumulative, as well as the time lag from current-year usage to exposure via the food chain, it is probable that tissue concentrations among the general population will continue to rise. The increasing temporal trend for PBDEs in human tissues is illustrated by findings of an exponential increase of PBDEs in Swedish human breast milk from 1972 to 1997 with a doubling rate of 5 years (Norén and Meironyté (1998, 2000). The milk concentrations of PBDEs (sum of eight congeners) on a lipid basis were 0.07 ppb in 1972 and 4 ppb in 1997 (Meironyté et al. 1999). Analysis of samples from 11 Finnish women showed that PBDE concentrations (sum of four congeners, lipid basis) were similar in breast milk and placenta, with ranges of 0.99–5.89 and 1.00–4.40 ppb, respectively (Strandman et al. 2000). The four highest sum concentrations were from women following their first childbirth. No PBPK models have been developed for PBDEs that could be used to quantitatively predict transfer of PBDEs via breast milk or across the placenta.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to PBBs and PBDEs are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by PBBs and PBDEs are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10 “Populations That Are Unusually Susceptible”.

3.8.1 Biomarkers Used to Identify or Quantify Exposure to PBBs and PBDEs

Polybrominated Biphenyls. PBBs are environmental contaminants found mainly, but not exclusively, in body tissues and fluids of populations with known exposure to PBBs. Because they are lipophilic and have long half-lives, certain PBB congeners preferentially accumulate in lipid-rich tissues, especially adipose, and are present in serum and human milk. Both serum and adipose PBB levels are indicators of exposure, but monitoring PBBs simultaneously in samples of both types is more reliable than in serum only. The serum/adipose partition ratios for groups of pregnant and nonpregnant Michigan women and chemical workers ranged between 1:140 and 1:260; the value for Michigan male farmers was 1:325–329 (Eyster et al. 1983). These values agree with those reported by other investigators for similar populations (Landrigan et al. 1979; Wolff et al. 1982). The importance of a dual determination of PBBs in serum and adipose can be illustrated with the following example. In a Michigan cohort, 70% of 839 subjects were identified as having had exposure by their serum PBB levels. When adipose tissue results were added, an additional 24% indicated exposure (Wolff et al. 1982). The partition ratio of .1:300 made the adipose limit of detection a more sensitive indicator of exposure, even though the limit of detection in adipose was one order of magnitude higher than in serum. Partition ratios below those reported from groups expected to be in equilibrium may indicate current or recent exposure (Anderson 1985).

Using an animal physiological compartment model scaled to humans by adjusting tissue volume, blood flow, and clearance and rate constant parameters, it was predicted that human intake of 9.8 g of 2,2',4,4',5,5'-hexabromobiphenyl from milk consumption over a 230-day period would result in peak tissue concentrations of 720 and 2.1 ppm in adipose and blood, respectively, at 8 months, and 443 and 1.3 ppm, respectively (Tuey and Matthews 1980). The elimination rate after 5 years would be 1.63 mg/day, the body burden would be 5.2 g, and the half-life would be 6.5 years. When a dose of 0.1 mg/day for 10 months was simulated, the excretion rate in a lean individual was estimated at 10.2 µg/day; overweight individuals had an excretion rate of 4.1 µg/day. PBB in adipose tissue from the lean and overweight subjects were predicted to be 2,769 and 1,103 ppb, respectively. PBB in serum would be 8.1 ppb in lean subjects and 3.2 ppb in overweight subjects. These predictions point to the importance of the percentage of body fat in the equilibrium dynamics of PBBs and indicate that because lean individuals have a smaller fat compartment, all of their body tissues will have higher concentrations of PBB than those in fatter individuals of the same exposure (Tuey and Matthews 1980). The assumptions on which the predictions are based do not reflect possible differences in fat and lean subjects

due to the way that PBBs are compartmentalized and/or excreted as a percent of the total body burden, or in decay rates due to differential partitioning.

As indicated above, PBBs are persistent chemicals due to their lipophilicity. Some studies have reported practically no change in serum PBB levels over a 12–18-month period (Wolff et al. 1979b) or over a 3-year period (Landrigan et al. 1979). The half-life of 6.5 years predicted by Tuey and Matthews (1980) is shorter than half-life values determined using sera data from the Michigan PBB cohort (Lambert et al. 1990; Michels Blanck et al. 2000b; Rosen et al. 1995). A median half-life of 12.0 years (95% CI 4–97 years) was estimated based on two serum measurements from 15 women ($20 years of age) with an initial serum PBB level of $5 ppb (Lambert et al. 1990). An analysis of 51 women ($18.8 years of age) and 112 men ($18.1 years of age) with at least two measurements 1 year apart and an initial PBB level of $20 ppb found a median half-life of 13.0 years (95% CI 6.3–infinite years) and 10.0 years (95% CI 6.7–20.0 years), respectively (Rosen et al. 1995). Based on a median half-life of 10.8 years (95% CI 9.2–14.7 years) for the entire group (163 persons, median PBB level 45.5 ppb), it was estimated that it will take more than 60 years for their PBB levels to fall below a detection limit of 1 ppb.

Determinants of PBB serum decay were investigated in a group of 380 Michigan women ($16 years of age) who had an initial PBB level of at least 2 ppb and at least two measurements taken when they were not pregnant (Michels Blanck et al. 2000b). The mean initial PBB level was 20.9 ppb (standard deviation 78.7), and the mean time between the first and last measurement was 4.2 years (range 16.0–75.2 years). A total of 109 women (29%) did not have a reduction in serum PBB over time. Assuming that PBBs reached equilibrium in the body before substantial amounts were eliminated and before the first serum measurements were taken, the entire body was modeled as a single compartment for PBBs with exponential decay. The median PBB half-life in the entire group was 13.5 years (95% CI 10.5–23.2 years). Subject-specific decay rate estimates were regressed on predictor variables including initial age, body mass index (BMI), smoking history, breast-feeding duration, and parity. The serum PBB decay rate was slower, resulting in a longer half-life, with higher initial PBB levels; women with initial PBB levels of <10 and >10 ppb had median half-lives of 12.9 and 28.7 years, respectively. The PBB decay rate was also slower ($p=0.03$) in women with an initial BMI above the median (BMI$23). Increasing number of pregnancies between the first and last measurement was also associated with slower decay, but the effect was of borderline statistical significance ($p=0.06$). Breast feeding as either a continuous variable or as categorized by tertiles of duration (<3, 3–9, or >9 months), age, and smoking were not associated with serum PBB decay.

The average concentration of PBBs (on an adipose basis and as hexabromobiphenyl) in pooled extracts of several hundred individual tissue samples collected in a statistically valid manner from all nine regions of the continental United States was 1–2 ppb (Lewis and Sovocool 1982). Chemical workers involved in the PBB manufacturing process had a median adipose PBB concentration of 6,000 ppb (range 400–350,500 ppb); Michigan male farmers and chemical workers not involved in the PBB manufacturing process had a median of 1,050 ppb (range 70,000–350,000 ppb) (Eyster et al. 1983).

A potential biomarker of exposure to PBBs is related to their effect on the thyroid gland. As discussed in Sections 3.2.2.2, Endocrine Effects, the thyroid gland is an unequivocal target of PBBs in animals, and evidence in humans is suggestive of a similar relationship. Effects in workers exposed to unspecified PBBs and/or decabromobiphenyl included increased serum thyrotropin, low or borderline low serum T_4, and increased thyroid antimicrosomal antibody titers (Bahn et al. 1980). A spectrum of thyroid effects has been observed in exposed rats, ranging from decreases in serum levels of serum T_4 and T_3 to histological and ultrastructural changes (Akoso et al. 1982b; Allen-Rowlands et al. 1981; Byrne et al. 1987; Gupta and Moore 1979; Kasza et al. 1978a; Meserve et al. 1992; Norris et al. 1975a, 1975b; NTP 1983; Sepkovic and Byrne 1984; Sleight et al. 1978; Werner and Sleight 1981). Additional studies could better characterize thyroid effects of PBBs in humans and develop specific correlations between levels and duration of exposure and alterations in serum T_4 and T_3 levels. These potential biomarkers are not specific to PBBs because PBDEs and other antithyroid agents can have similar effects.

Polybrominated Diphenyl Ethers. PBDEs are also persistent environmental contaminants that accumulate in adipose tissue, serum, and breast milk serum of the general population due to their lipophilic characteristics. PBDEs in breast milk are useful markers of maternal body burdens as well as lactational and *in utero* exposures. The predominant congeners identified in milk and other human tissues are 2,2',4,4'-tetraBDE (BDE-47), 2,2'4,4',5-pentaBDE (BDE-99), and 2,2'4,4',5,5'-hexaBDE (BDE-153) (all *ortho-para* substituted congeners). Considering the continued widespread production and use of PBDEs, particularly commercial pentaBDE mixtures whose tetra- to hexaBDE congeners are highly bioaccumulative, as well as the time lag from current-year usage to exposure via the food chain, it is highly likely that tissue concentrations among the general population will continue to rise. The increasing temporal trend for PBDEs in human tissues is suggested by findings of an exponential increase of PBDEs in Swedish human breast milk from 1972 to 1997 with a doubling rate of 5 years (Norén and Meironyté (1998, 2000). In the 1997 sample, the concentration of PBDE (sum of eight congeners) was 4 ppb on a lipid basis, whereas the 1972 sample contained 0.07 ppb (Meironyté et al. 1999). PBDEs have been detected in human placenta at concentrations similar to those in breast milk from the same women;

concentrations (sum of four congeners, lipid basis) ranged from 0.99 to 5.89 ppb in milk and from 1.00 to 4.40 ppb in placenta (Strandman et al. 2000).

Estimates of PBDE serum concentrations among electronics-dismantling workers before and after exposure-free vacation (median duration 28 days, range 21–35 days) indicate that the higher brominated congeners have shorter half-lives than lower brominated congeners (Sjödin et al. 1999b). The median percentage decrease in serum concentrations, based on 5–11 measurements per congener, were 14 (range 3.5–39), 14 (2.1–38), 14 (6.7–42), 30 (7.9–52), and 66 (47–100) for 2,2',4,4'-tetraBDE (BDE-47), 2,2',4,4',5,5'-hexaBDE (BDE-153), 2,2',4,4',5,6'-hexaBDE (BDE-154), 2,2',3,4,4',5',6-heptaBDE (BDE-183), and 2,2',3,3',4,4',5,5',6,6'-decaBDE (BDE-209), respectively. Although actual half-lives were not calculated, the data suggest that the half-lives of the lower brominated congeners were <1 year.

Congener patterns in humans may provide information on the nature or pathway of PBDE exposures (Hooper and McDonald 2000). Low tetra:deca congener ratios are suggestive of direct, recent, or occupational exposures to the parent PBDE mixture, whereas higher ratios may indicate an environmental pathway where exposures result from PBDEs that have leached from the parent mixtures and have been degraded in the environment.

A potential biomarker of exposure to PBDEs relates to their effect on the thyroid gland. As discussed in Sections 3.2.2.2, Endocrine Effects, thyroid changes in rats and mice include reduced serum thyroxine (T_4) levels with no changes in serum TSH (Darnerud and Sinjari 1996; Fowles et al. 1994; Hallgren and Darnerud 1998;WIL Research Laboratories 1984; Zhou et al. 2001, 2002). Additional studies are needed to characterize thyroid effects of PBDEs in humans and develop specific correlations between levels and duration of exposure and alterations in serum levels of T_4. This potential biomarker is not specific to PBDEs because PBBs and other antithyroid agents can have similar effects.

3.8.2 Biomarkers Used to Characterize Effects Caused by PBBs and PBDEs

Polybrominated Biphenyls. A recent study has used caffeine as a potential tool to characterize exposure and/or effect of PBBs (Lambert et al. 1990). In this test, caffeine is used as a metabolic probe of cytochrome P-450 isozymes activity from the CYP1A family, which in animals is significantly induced by PBBs (Safe 1984). The caffeine breath test (CBT) is primarily useful for detecting induction of CYP1A2 activity in human liver, and for that reason, it also has been used as a marker for exposure to PCBs, CDDs, and CDFs (Lambert et al. 1992). A volunteer population of 50 Michigan subjects with previously high serum PBB levels and 50 with undetectable or low serum levels was compared to a control population not exposed to PBBs (Lambert et al. 1992). Two tests were conducted, the CYP1A2-dependent caffeine 3-N-demethylase activity was monitored by the CBT, and 7-N-demethylase activity was monitored by the caffeine urinary metabolite ratio (CMR). PBB-exposed subjects had higher CBT values ($p<0.02$) than urban nonsmokers, but the values were comparable to those of urban smokers. The correlation between serum PBB levels and the CBT value was poor ($r^2=0.2$). The CMR value in PBB-exposed subjects was also higher than that of urban nonsmokers ($p<0.05$); there was no correlation between serum PBB levels and CMR values. Generally, smokers have higher CBT values than nonsmokers due to the presence of polycyclic aromatic hydrocarbons (PAH) in tobacco smoke, which induce CYP1A (Kotake et al. 1982).

The thyroid gland is a sensitive target of PBBs. As discussed in Section 3.2.2.2, Endocrine Effects, effects in exposed workers included increased serum thyrotropin, low or borderline low serum T_4, and increased thyroid antimicrosomal antibody titers (Bahn et al. 1980). Thyroid effects in PBB-treated rats included reduced serum levels of serum T_4 and T_3 (Akoso et al. 1982b; Allen-Rowlands et al. 1981; Byrne et al. 1987; Gupta and Moore 1979; Kasza et al. 1978a; Meserve et al. 1992; Norris et al. 1975a, 1975b; NTP 1983; Sepkovic and Byrne 1984; Sleight et al. 1978; Werner and Sleight 1981). Therefore, serum levels of T_4 and/or other thyroid hormones are potential biomarkers of effect for PBBs. Although information is needed on the specific amount of change in these biomarkers associated with a demonstrably adverse effect, changes in these parameters might indicate potential impairment of health.

Many reports have been published regarding possible associations between PBB exposure and adverse health effects in populations from the state of Michigan. An early study compared the health status of people on quarantined farms with people in nonquarantined farms in the same area (Humphrey and Hayner 1975). The results showed no pattern of differences between the groups. Moreover, no abnormalities of heart, liver, spleen, or nervous system that could be related to PBB exposure were found

in physical examinations. A follow-up study examined the prevalence of selected symptoms in groups of varying potential exposure 4 years after exposure (Landrigan et al. 1979). In general, symptoms were more prevalent in two self-selected groups and were least prevalent in the group composed of chemical workers. No positive associations were found between serum PBB concentrations and symptom frequencies; yet a third group of studies reported an increased incidence of symptoms in Michigan farmers relative to a group of control Wisconsin farmers (Anderson et al. 1978a, 1978b, 1978c, 1979). As observed in other epidemiology studies, self-selected groups, which had lower PBB concentrations in serum, reported a high incidence of symptoms, compared to randomly selected groups. No specific biomarker of effect could be identified in the Michigan contamination episode. Furthermore, the prevalence of the reported symptoms had no consistent relationship to the extent or types of exposure, and most objective clinical measurements have failed to show a significant relationship to PBB exposure (Fries 1985a). Additional information regarding biomarkers for effects can be found in OTA (1990) and CDC/ATSDR (1990). For a more detailed discussion of the health effects caused by PBBs see Section 3.2.

Polybrominated Diphenyl Ethers. The thyroid is a critical target for PBDEs in animals and, based on these data (see Section 3.2.2.2 Endocrine Effects), serum T_4 level is a potential biomarker of effect in humans. Although this biomarker is not specific to PBDEs because other antithyroid agents can have similar effects, changes in T_4 can be considered to indicate potential impairment of health.

3.9 INTERACTIONS WITH OTHER CHEMICALS

Polybrominated Biphenyls. PBBs are potent inducers of liver and kidney P-450 enzymes (MFO) (Haake et al. 1985; Halvorson et al. 1985; Shepherd et al. 1984), and as such, they could potentially enhance or decrease the toxicity of any substance that is metabolized by the P-450 system. PBBs are thought to potentiate the hepatotoxicity and nephrotoxicity of halogenated hydrocarbons and other substances by inducing P-450s that biotransform them to more toxic metabolites (Ahmadizadeh et al. 1984; Kluwe and Hook 1978; Kluwe et al. 1978, 1979, 1982; Kuo and Hook 1982; Roes et al. 1977). In these studies, rats and/or mice were given diets containing FireMaster BP-6 that provided doses of 0.13–13 mg/kg/day for periods of 10–28 days prior to intraperitoneal challenge with the halogenated hydrocarbons. Nephrotoxicity was assessed by measuring kidney weights and the levels of blood urea nitrogen, and by the accumulation of *p*-aminohippurate and/or tetraethylammonium (TEA) in renal cortical slices. Hepatotoxicity was assessed by relative liver weights and by levels of SGPT and/or SGOT. In most cases, exposure to PBBs alone did not affect the parameters of nephrotoxicity in animals. However,

exposure to PBBs alone usually caused increased relative liver weights and elevated levels of SGOT and SGPT. Pre-exposure to PBBs increased the hepatotoxicity and nephrotoxicity of chloroform (Ahmadizadeh et al. 1984; Kluwe and Hook 1978; Kluwe et al. 1978) and carbon tetrachloride (Kluwe et al. 1979, 1982) and the nephrotoxicity of trichloroethene and 1,1,2-trichloroethane (Kluwe et al. 1978, 1979). PBB pretreatment in dietary studies also potentiated the nephrotoxicity of the antibiotic, cephaloridine, in rats (Kuo and Hook 1982).

Pretreatment with PBBs also potentiated the lethality of chloroform, carbon tetrachloride, and 1,1,2-trichloroethane by decreasing the LD_{50} values (Kluwe et al. 1978, 1979) and the lethality of bromobenzene by decreasing the time to death (Roes et al. 1977) in mice after challenge with the halogenated hydrocarbon. In contrast, pretreatment of mice with PBBs in dietary studies increased the LD_{50} value of 1,2-dibromo-3-chloropropane (DBCP) but had no effect on the LD_{50} value of 1,2-dibromoethane (EDB) (Kluwe et al. 1981). Also, PBBs were found to reverse the depletion of nonprotein sulfhydryls (e.g., glutathione) caused by DBCP and EDB in the livers and kidneys of mice, suggesting that PBB exposure protected the mice from the lethality of DBCP by making glutathione more available for conjugation with the toxic metabolites.

No potentiation of toxicity was found when rats were co-exposed to diets containing PBB and mirex, photomirex, or kepone, compared with toxicity elicited by each of these substances alone (Chu et al. 1980).

Polybrominated Diphenyl Ethers. No specific information was located regarding interactions between PBDEs and other chemicals. PBDEs are inducers of hepatic microsomal enzymes (Carlson 1980a, 1980b; Fowles et al. 1994; Hallgren et al. 2001; Zhou et al. 2001, 2002) and therefore could potentially enhance or decrease the toxicity of any substance that is metabolized by the P-450 system.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to PBBs and PBDEs than will most persons exposed to the same level of PBBs and PBDEs in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of PBBs and PBDEs, or compromised function of organs affected by PBBs and PBDEs. Populations who are at greater risk due to their

unusually high exposure to PBBs and PBDEs are discussed in Section 6.7, Populations With Potentially High Exposures.

Limited information was located on populations that may be unusually susceptible to PBBs and PBDEs. As indicated in Section 3.4.4.2, breast milk constitutes the most important route of excretion of PBBs in lactating females, and this is likely to be the case for PBDEs as well. Therefore, women with high body burdens of PBBs and/or PBDEs who breast-feed may be placing their infants at a higher risk of potential health effects. Experiments in animals and model simulations in humans have shown that reduction in body fat markedly decreases the elimination half-life of PBBs (Domino et al. 1982; Tuey and Matthews 1980). For example, when a dose of 0.1 mg/day for 10 months was simulated in humans, the excretion rate in a lean individual was estimated at 10.2 µg/day; overweight individuals had an excretion rate of 4.1 µg/day. The cumulative excretion was 51% of the dose in lean subjects compared to 20.7% in overweight subjects. These data indicate that overweight individuals may be at higher risk because they store PBBs for a longer time than lean subjects. On the other hand, because lean individuals have a smaller fat compartment, their body tissues will contain higher concentrations of PBB than those in subjects with more fat who received the same exposure (Tuey and Matthews 1980); thus, leaner individuals may be more vulnerable to short-term effects than fatter individuals. Because of this phenomenon, a sudden reduction in body fat, such as that which could occur during dieting, may cause a redistribution of PBBs to potential target organs, which would also increase the potential for adverse health effects to such individuals.

Pregnant women and developing infants and fetuses should be viewed as sensitive populations for exposure to PBBs, PBDEs, and other thyroid hormone disrupting chemicals (Glinoer 1990; McDonald 2002; Morreale de Escobar et al. 2002). The condition of pregnancy normally puts a significant strain on the maternal thyroid system, which can be exacerbated by iodine deficiency; according to data from 1988 to 1994, iodine deficiency is prevalent in approximately 12% of the general population and 15% of women of child-bearing age in the United States (Hollowell et al. 1998). Thyroid hormones are essential for normal development of the nervous system, lung, skeletal muscle, and possibly other organ systems, and the fetus is dependent on maternal thyroid hormones at least until the fetal thyroid begins to produce T_4 and T_3, which occurs in humans at approximately 16–20 weeks of gestation (Zoeller and Crofton 2000). The potential of PBDEs to disrupt maternal, fetal, and newborn thyroid hormone levels is demonstrated by the Zhou et al. (2001, 2002) studies of pentaBDE (technical mixture) in rats as discussed in Section 3.2.2.2 (Endocrine Effects subsection).

People with exposure to anti-thyroid drugs (e.g., lithium), thyroid disease, or otherwise compromised thyroid function might have a more pronounced response to PBBs and PBDEs because of their underlying limitations in thyroid hormone production. Similarly, people with compromised function of other organs, such as those with liver or kidney diseases (e.g., liver cirrhosis or hepatitis B), could be considered more susceptible to health effects of PBBs and PBDEs.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to PBBs and PBDEs. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to PBBs and PBDEs. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. No texts were found that provided specific information about treatment following exposures to PBBs and PBDEs.

3.11.1 Reducing Peak Absorption Following Exposure

Ingested PBBs and PBDEs are absorbed by the gastrointestinal tract of humans and animals (see Section 3.4). Although specific recommendations for clinical treatment of acute intoxication from ingested PBBs and PBDEs were not located, recommendations based on experiences with PCBs are relevant. Treatments for acute poisonings from PCBs and related substances include the induction of emesis or gastric lavage and stomach pumping to decrease gastrointestinal absorption of the chemicals (Lemesh 1992). These procedures would not be beneficial if performed too long after exposure occurred. Administration of activated charcoal as a slurry, either aqueous or mixed with a saline cathartic or sorbitol, is frequently recommended to decrease the gastrointestinal absorption of PCBs, but the value of this treatment for reducing absorption of PCBs, PBBs, and PBDEs is unknown (HSDB 1992). Repetitive administration of activated charcoal might be useful in preventing reabsorption of metabolites. Rice bran fiber decreased absorption of PCBs in the gastrointestinal tract and had a stimulatory effect on fecal excretion of PCBs in rats (Takenaka et al. 1991), but it is unclear if rice bran would be of benefit in poisoned humans.

The detection of PBBs and PBDEs in the serum and fat of people who were occupationally exposed to these chemicals indicates that PBBs and PBDEs can also be absorbed by the lungs and skin in humans. Although no specific methods to reduce absorption of dermally applied or inhaled PBBs or PBDEs were

located, multiple washings of contaminated skin with soap and water immediately following exposure have been suggested to reduce the dermal absorption of PCBs (HSDB 1992). Studies with monkeys showed that soap and water was as effective as or better than such solvents as ethanol, mineral oil, or trichlorobenzene in removing PCBs from skin (Wester et al. 1990).

3.11.2 Reducing Body Burden

PBBs and PBDEs tend to accumulate in lipid-rich tissues and are slowly metabolized and eliminated from the body (see Section 3.4). Several methods to enhance the elimination of PBBs from the body have been examined in animals and are also relevant to PBDEs, including the restriction of caloric intake (to reduce total body fat), and the administration of various agents that interact with bile acids including activated charcoal, mineral oil and bile-binding resins such as cholestyramine (Kimbrough et al. 1980; McConnell et al. 1980; Polin and Leavitt 1984; Polin et al. 1985, 1991; Rozman et al. 1982). It should be mentioned, however, that based on the pharmacokinetic considerations discussed in Section 3.8.1, a rapid breakdown of fat, as might occur in dieting, might lead to a transient increase in PBB and PBDE levels in serum and other body tissues, possibly posing a significant re-exposure problem. Although some of the studies observed no enhanced elimination (Kimbrough et al. 1980; McConnell et al. 1980), others identified treatments that were effective in enhancing the biliary and intestinal elimination of PBB residues (Polin et al. 1991; Rozman et al. 1982). Polin et al. (1991) found that dietary intervention to reduce PBBs was dose dependent; treatment with 10% mineral oil and a 45% reduction in food intake resulted in a 69 and 23% reduction in body burden in rats fed PBBs at dietary concentrations of 0.1 and 100 ppm, respectively (Polin et al. 1991). A combination of mineral oil, colestipol, and dietary restriction was successful in reducing the PBB body burdens in chickens (Polin and Leavitt 1984; Polin et al. 1985), while each treatment alone had no effect in reducing PBB body burden. A 3-week treatment regimen that included dietary supplements of polyunsaturated oil, vitamins, and minerals, and heat stress has been applied in a pilot study to seven human subjects that were known to have been exposed to PBBs; following treatment, statistically significant reductions were measured in PBB concentrations in fat (Schnare et al. 1984). Although the lack of a separate control group complicates interpretation of the results of this study (each subject served as his/her own control), this treatment was developed for the purpose of reducing body burdens of fat-soluble psychoactive drugs (Schnare et al. 1984). A liquid diet was used for 16 individuals who developed symptoms following exposure to PCBs and polychlorinated dibenzofurans (Imamura and Tung 1984). Symptoms were reduced several months after the fasting period. This study is limited in that a control group was not used, and body burdens were not measured. Mobilization of PBBs or PBDEs from adipose tissue is not recommended in individuals with hepatic or renal disease (Lemesh 1992).

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

There are no known methods for interfering with the mechanism of action of PBBs or PBDEs. Although the mechanism of action of PBBs and PBDEs is not completely understood, experimental evidence accumulated in recent years indicates that some PBB and PBDE congeners exert toxic actions by a process involving several steps (Safe 1984). This process begins with the binding of particular congeners to the Ah receptor and leads ultimately to enhancement of the CYP1A1 gene expression (see Section 3.5). It appears, therefore, that interfering with the initial step, binding to the receptor, or with any of the subsequent steps, would possibly prevent the expression of the toxic effects. It must be mentioned that several compounds have been identified that partially antagonize one or more Ah receptor-mediated responses (Bannister et al. 1989); their use, however, has been limited to experimental studies in animals. These compounds were successful antagonists when given before or at the same time as an Ah receptor activator (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) (Bannister et al. 1989). Therefore, the potential for interfering with the mechanism of PBB/PBDE effects after exposure has occurred is largely untested.

PBBs and PBDEs may also cause toxicity by other mechanisms of action. For example, some PBB congeners can be metabolized to reactive arene oxides (Kohli and Safe 1976; Kohli et al. 1978) that may alkylate critical cellular macromolecules and result in injury. PBDEs may disrupt thyroid hormones by induction of hepatic microsomal UDPGT, which increases the rate of T_4 conjugation and excretion, or by mimicking T_4 or T_3; PBDEs and their hydroxy metabolites are structurally similar to these thyroid hormones which are also hydroxy-halogenated diphenyl ethers (see Section 3.5.2). Clinical interventions designed to interfere with this mechanism or the metabolism of PBBs have yet to be developed.

3.12 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of PBBs and PBDEs is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of PBBs and PBDEs.

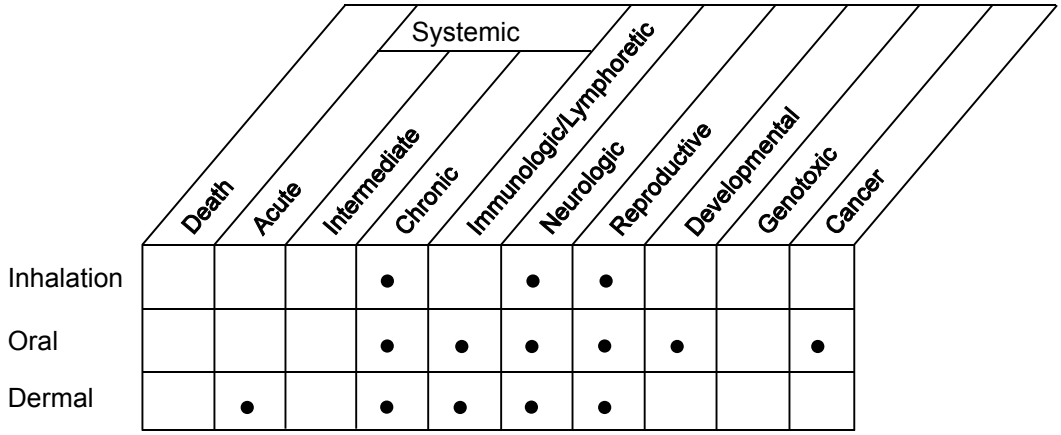
The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

3.12.1 Existing Information on Health Effects of PBBs and PBDEs

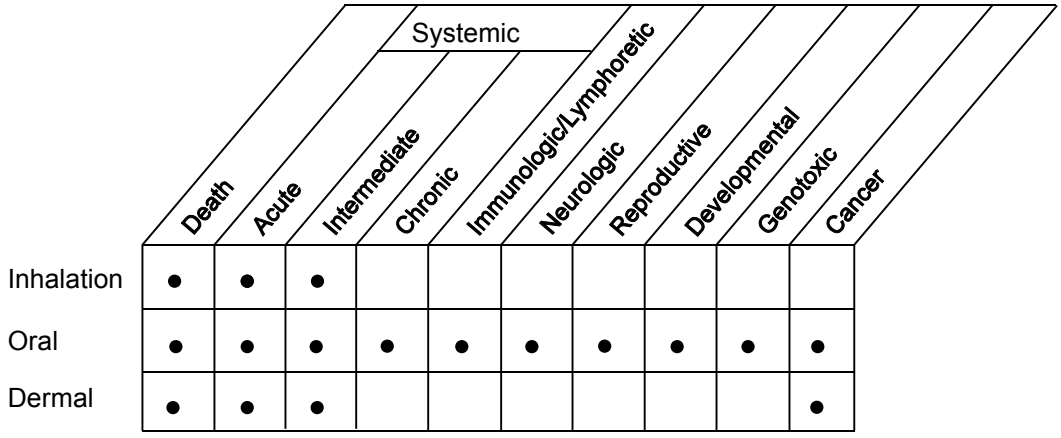
The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to PBBs and PBDEs are summarized in Figures 3-4 and 3-5, respectively. The purpose of this figure is to illustrate the existing information concerning the health effects of PBBs and PBDEs. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

As indicated in Figure 3-4, information is available regarding systemic, immunological, neurological, developmental, reproductive, and carcinogenic effects of PBBs in humans. The information on effects in humans is derived from the Michigan contamination episode that involved chronic-duration oral exposure to contaminated food and from occupational exposure data in which it was assumed that exposure was predominantly through skin contact, although inhalation exposure cannot be ruled out.

Figure 3-4. Existing Information on Health Effects of PBBs



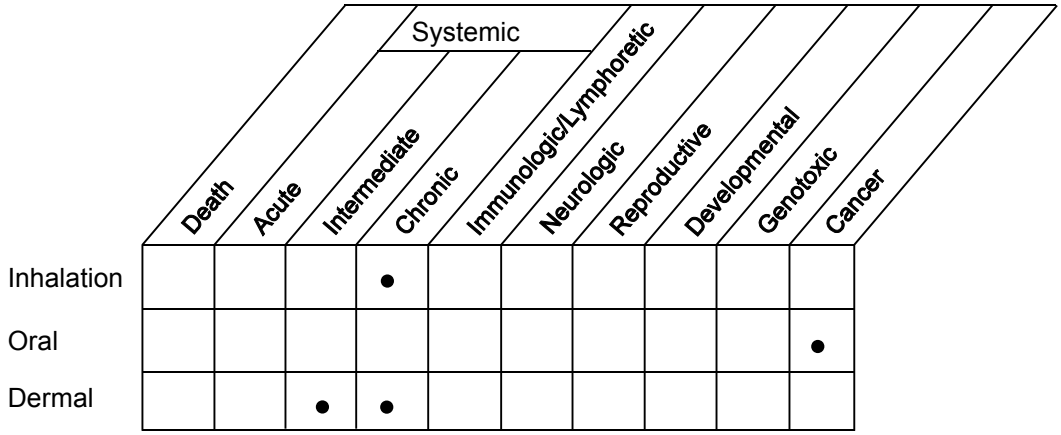
Human



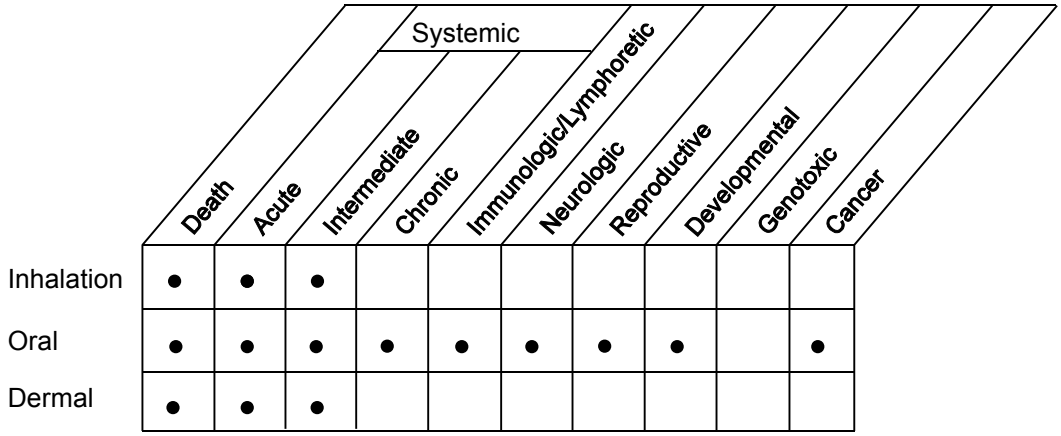
Animal

- Existing Studies

Figure 3-5. Existing Information on Health Effects of PBDEs



Human



Animal

● Existing Studies

Information on health effects in animals is extensive and available for all effect categories, but is nearly completely limited to oral exposure studies, which appears to reflect experimental practicality and concern for what is thought to be the most prevalent and likely route of environmental exposure.

A limited amount of information is available on the systemic and carcinogenic effects of PBDEs in humans (Figure 3-5). Information on health effects of PBDEs in animals is available for all effect categories but, like PBBs, is mainly limited to oral exposure studies. In general, the health effects of PBDEs are less adequately studied than for PBBs (and PCBs).

3.12.2 Identification of Data Needs

Acute-Duration Exposure.

Polybrominated Biphenyls. The hepatotoxicity of PBBs in rats and mice is reasonably well characterized for acute-duration oral exposure (Bernert et al. 1983; Corbett et al. 1975; Gupta and Moore 1979; Gupta et al. 1981; Kimbrough et al. 1978b, 1980, 1981; Lee et al. 1975a, 1975b; Norris et al. 1975a; Raber and Carter 1986; Waritz et al. 1977). Effects on body weight in rats and mice and on the thyroid in rats are also well documented (Allen-Rowlands et al. 1981; Corbett et al. 1978; Fraker 1980; Gupta and Moore 1979; Kimbrough et al. 1981), and thyroid effects occurred at doses as low as those causing liver effects. Insufficient acute data exist to definitely establish if the thyroid effects are more critical than effects in the liver, but extensive data on thyroid effects from longer term studies and the functional nature of the changes suggest that this is the case and justify using a thyroid effect as the basis for an acute oral MRL. Acute oral studies in other species would clearly establish the most sensitive target and species for acute exposure. Tests with monkeys, guinea pigs, and mink would be informative because intermediate- and chronic-duration studies indicate that these species are more sensitive than the rat and that endocrinological effects are particularly sensitive end points.

Information on toxic effects of acute-duration exposure to PBBs by routes other than oral are limited to data on hepatic, renal, dermal, and ocular effects of inhalation and dermal exposure in rats or rabbits (Millischer et al. 1980; Needham et al. 1982; Norris et al. 1975a; Waritz et al. 1977), but these data may not be reliable due to study limitations and possible delayed lethality. Quantitative data for inhalation and dermal absorption of PBBs are lacking. Studies of inhalation and dermal absorption following exposure to soil containing PBBs (i.e., bioavailability studies) would be useful for assessing risk at a hazardous waste site. Further studies identifying target organs and examining the dose-response relationship

following acute inhalation and dermal exposure to PBBs would also be informative, although exposure via soil and acute toxicosis is not likely to ever be a concern.

Polybrominated Diphenyl Ethers. Acute-duration studies have documented effects of PBDEs mainly on the liver, thyroid, and body weight of orally exposed rats and mice (Argus Research Laboratories 1985a; Carlson 1980a, 1980b; Darnerud and Sinjari 1996; Fowles et al. 1994; Hallgren et al. 2001; IRCD 1974, 1975a, 1975b; Life Science Research Israel Ltd. 1987; NTP 1986; WIL Research Laboratories 1986; Zhou et al. 2001, 2002). The available data indicate that the thyroid is a particularly sensitive target of acute oral exposure and justify using thyroid effects as the basis for an acute oral MRL, but acute effects of PBDEs on the liver are not as well characterized as thyroid effects. Other studies indicate that immunosuppression and neurobehavior are important and potentially critical health end points for acute exposure to PBDEs that need to be further investigated (see discussions of data needs for Immunotoxicity and Neurotoxicity). Studies in other species would help to clearly establish the most sensitive target and species for acute exposure, as well as which animal toxicity data are the most relevant to humans and useful for assessing acute health risks.

Intermediate-Duration Exposure.

Polybrominated Biphenyls. The preponderance of toxicity data for PBBs are available from animals exposed to FireMaster FF-1 or FireMaster BP-6 in the diet or by gavage in intermediate-duration studies (Akoso et al. 1982a, 1982b; Allen et al. 1978; Allen-Rowlands et al. 1981; Aulerich and Ringer 1979; Byrne et al. 1987, 1988; Castracane et al. 1982; Darjono et al. 1983; Gupta and Moore 1979; Gupta et al. 1981; Kasza et al. 1978a, 1978b; Ku et al. 1978; Lambrecht et al. 1978; Lee et al. 1975a, 1975b; Loose et al. 1981; McCormack et al. 1978; Norris et al. 1975a; NTP 1983; Ringer et al. 1981; Sepkovic and Byrne 1984; Sleight and Sanger 1976; Sleight et al. 1978; Waritz et al. 1977; Werner and Sleight 1981). Studies have been performed with various species (rats have been tested most extensively), and there is evidence indicating that monkeys, guinea pigs, and mink may be the most sensitive. The liver, skin, stomach, and thyroid are unequivocal targets, but existing studies do not identify NOAELs for toxic effects in these organs in rats and/or more sensitive species. Hematologic changes indicative of anemia are consistently reported effects in various species, but the relative importance of these effects is not known. Evidence suggests that the LOAELs for thyroid effects in rats and hepatic effects in guinea pigs are similar (Akoso et al. 1982b; Sleight and Sanger 1976), but reproductive and developmental effects occurred in monkeys at a lower dosage. The serious nature of the developmental toxicity (fetal death) precludes derivation of an intermediate-duration oral MRL. Additional intermediate-duration dose-response studies determining

NOAELs for the most sensitive end points, as well as the most sensitive species, would be useful for possible MRL derivation. Studies addressing interspecies differences could help to better characterize the relative sensitivity of monkeys and humans, particularly the possibility that monkeys are more sensitive than humans, as indicated by the high reproductive/developmental toxicity of PPBs in this species that has not been noted in PBB-exposed workers or the Michigan cohort. These studies could also help elucidate the toxicological significance of effects in the thyroid and other endocrine organs, particularly since the reproductive effects may be related to endocrine imbalance.

Limited information is available on effects of PBBs in animals by inhalation or dermal exposure for intermediate durations (Millischer et al. 1980; Norris et al. 1975a; Waritz et al. 1977). Some inhalation data are available for octabromobiphenyl and decabromobiphenyl mixtures and some dermal data are available for octabromobiphenyl mixture, but intermediate-duration inhalation and dermal studies of FireMaster PBBs have not been performed. Studies of FireMaster FF-1 or FireMaster BP-6 would be particularly useful because these are likely to be the most toxic PBBs based on oral data and due to their higher content of potentially toxic congeners. Although the octabromobiphenyl mixture inhalation data are limited by numbers of animals, dose levels, and end points, and only one species (rat) was tested in the octabromobiphenyl mixture and decabromobiphenyl inhalation studies, it appears that these PBB mixtures are not highly toxic. Due to the inadequacies of the octabromobiphenyl mixture data and lack of any information on inhalation toxicity of the likely more potent FireMaster mixtures, there is insufficient basis for deriving an intermediate inhalation MRL. Although intermediate-duration inhalation studies of FireMaster PBBs would be particularly relevant to MRL derivation, they may not be practical due to the low volatilization potential of PBBs. The intermediate-duration dermal studies of octabromobiphenyl mixtures revealed some skin irritation in rabbits but no sensitization in guinea pigs. Additional studies could corroborate the potential for dermal irritation by PBBs and are relevant because the skin is a route of concern for exposure at or near hazardous waste sites. Intermediate-duration inhalation and dermal exposure studies of PBB-contaminated soil (e.g., bioavailability studies) that identify thresholds would be especially useful for risk assessment at a hazardous waste site.

Polybrominated Diphenyl Ethers. Available intermediate-duration oral studies in animals indicate that the liver and thyroid are the main systemic targets of repeated exposures to PBDEs as shown by effects that mainly include enlargement and histological alterations in both organs and changes in serum levels of thyroid hormones, particularly decreases in serum T_4 (Carlson 1980a; IRDC 1976, 1977; Norris et al. 1973, 1975a, 1975b; WIL Research Laboratories 1984; Zhou et al. 2001, 2002). Hepatic effects occurred at the lowest LOAEL and were used as the basis for an intermediate oral MRL, but thyroid effects

occurred at doses as nearly as low as those causing liver effects and the data are insufficient to clearly characterize liver effects as more critical than effects in the thyroid. Studies designed to identify NOAELs for deca-, octa-, and pentaBDE could provide a better basis for an intermediate duration MRL, as well as help to ascertain the most appropriate PBDE mixture on which to base a chronic MRL. Additionally, mechanistic studies indicate that PBDE-induced serum T_4 depression may be related to hepatic enzyme induction. Studies designed to better characterize the mechanism of action of PBDEs would help to better understand how the animal toxicity data can best be used to identify target end points and assess health risks in humans.

Chronic-Duration Exposure and Cancer.

Polybrominated Biphenyls. Information on chronic systemic toxicity of PBBs in animals is limited to an oral bioassay showing hepatic, gastric, hematologic, and/or thyroid effects in rats and mice (NTP 1992), and a study showing effects on skin, stomach, and body weight in two monkeys (Allen et al. 1979; Lambrecht et al. 1978). Although limited by the number of studies and species, the available chronic animal data corroborate the results of intermediate-duration studies with respect to the observed effects. Additional studies would be necessary to determine the most sensitive animal target organ and species for chronic exposure and to provide a basis for an MRL, as serious hepatic changes as well as weight loss, decreased survival, and developmental effects occurred at the lowest tested dosages. Because PBBs are no longer being produced, exposure is most likely to occur at a contaminated waste site. Therefore, chronic studies examining the effects of PBB-contaminated soil following oral, inhalation, and dermal exposure (i.e., bioavailability studies) would be particularly useful. Evaluations of the thyroid would be particularly informative because intermediate-duration animal studies indicate that the thyroid may be a particularly sensitive target organ.

There is sufficient evidence that commercial hexabromobiphenyl mixtures (FireMaster FF-1) are hepatocarcinogenic in rats and mice following acute, intermediate, and/or chronic exposure (Groce and Kimbrough 1984; Kimbrough et al. 1978b; NTP 1983, 1992). Additional animal studies could provide useful information on interspecies differences and carcinogenesis of other PBB mixtures.

Polybrominated Diphenyl Ethers. Information on the chronic toxicity and carcinogenicity of PBDEs is limited to two oral studies of decaBDE mixtures. In one of the studies, rats were fed a 77.4% pure decaBDE mixture (containing 21.8% nonaBDE and 0.8% octaBDE) at doses of 0.01–1 mg/kg/day for approximately 2 years (Kociba et al. 1975; Norris et al. 1975a). Evaluations that included clinical signs,

body weight, food consumption, hematology, clinical chemistry, urine indices, and comprehensive histological examinations showed no exposure-related effects. In the other chronic study, decaBDE (94–97% pure) was fed to rats in doses of 1,120–2,550 mg/kg/day and mice in doses of 3,200–7,780 mg/kg/day for 103 weeks (NTP 1986). Histopathological changes occurred in both species that included liver effects in rats and liver and thyroid effects in mice. The highest chronic NOAELs in these studies are 1 mg/kg/day for the decaBDE/nonaBDE mixture (Kociba et al. 1975; Norris et al. 1975a) and 1,120 mg/kg/day for decaBDE (NTP 1986). Neither of these NOAELs is appropriate for estimation of a chronic oral MRL for all PBDEs because MRLs derived from these studies would be higher than the intermediate-duration oral MRL. The relative insensitivity of the chronic studies appears to be related to low potency of decaBDE compared to the pentaBDE mixture tested in the critical intermediate-duration study.

Neoplastic effects in the NTP (1986) bioassay included increased incidences of neoplastic nodules in the liver in the male and female rats and hepatocellular adenoma or carcinoma (combined) in the male mice. Slightly elevated incidences of thyroid gland follicular cell tumors were additionally observed in exposed male mice, although the increases were equivocal. No exposure-related neoplastic changes were found in the chronic study of the 77.4% decaBDE mixture (Kociba et al. 1975; Norris et al. 1975a), but the power of this study to detect carcinogenic effects is limited by the very low dose levels in comparison to those tested in the NTP bioassay.

Considering the insensitivity of the decaBDE studies for MRL derivation, as well as the lack of cancer information on the lower brominated PBB mixtures that may have higher carcinogenic potency than decaBDE, there is a need for chronic toxicity studies on pentaBDE and/or octaBDE. Evaluations that include the thyroid would be particularly informative because the acute and intermediate-duration animal studies indicate that the thyroid is a particularly sensitive target organ for PBDEs.

Genotoxicity.

Polybrominated Biphenyls. No information is available regarding potential genotoxic effects of PBBs in exposed humans. PBB mixtures or congeners were not genotoxic in any of the prokaryotic or eukaryotic animal systems tested. These include *in vitro* assays with *S. typhimurium* and *E. coli* bacteria (Haworth et al. 1983; Millischer et al. 1980; NTP 1983; Rossman et al. 1991), a host-mediated assay with *S. typhimurium* (Millischer et al. 1980), and *in vitro* assays with hamster cells (Galloway et al. 1987; Kavanagh et al. 1985; Williams et al. 1984), rat liver cells (Kavanagh et al. 1985; Williams et al. 1984),

mouse liver and lymphoma cells (Myhr and Caspary 1991; Williams et al. 1984), and human fibroblasts (Williams et al. 1984). PBBs also were inactive in *in vivo* unscheduled DNA synthesis assays with rat and mouse hepatocytes (Mirsalis et al. 1985, 1989) and in a micronucleus test with mice (Millischer et al. 1980). However, only some of these studies tested commercial PBB mixtures (Kavanagh et al. 1985; Millischer et al. 1980; Myhr and Caspary 1991; NTP 1983; Rossman et al. 1991; Williams et al. 1984). Additional studies of commercial mixtures could more fully characterize the genotoxic potential of PBBs, and provide information regarding differences in potencies of different mixtures and the sensitivities of different organisms. Cytogenic analysis of human populations exposed to PBBs in occupational settings, or exposed by consumption of food contaminated with PBBs, might make it possible to more adequately assess the genotoxic potential of these compounds in humans.

Polybrominated Diphenyl Ethers. A limited amount of information has been published on the genotoxicity of PBDEs. Cytogenetic examination of bone marrow cells showed no increase in aberrations in maternal and neonatal rats following maternal oral exposure to a 77.4% decaBDE mixture (containing 21.8% nonaBDE and 0.8% octaBDE) for 90 days prior to mating and during mating, gestation, and lactation (Norris et al. 1973, 1975a). *In vitro* assays found that decaBDE did not induce gene mutations in bacteria (*S. typhimurium*) or mammalian cells (mouse lymphoma L5178Y cells), and did not induce sister chromatid exchange or chromosomal aberrations in Chinese hamster ovary cells (NTP 1986). *In vitro* exposure to the single congeners 2,2',4,4'-tetraBDE, 3,4-diBDE, and 2-monoBDE caused increased recombinogenic activity in Chinese hamster SPD8 and Sp5V79 cells (Helleday et al. 1999). Although the weight of available evidence indicates that decaBDE is not genotoxic, studies using lower brominated mixtures and a wider variety of assay types would help to better characterize the genotoxic potential of PBDEs.

Reproductive Toxicity.

Polybrominated Biphenyls. A limited amount of information is available regarding reproductive effects in humans after exposure to PBBs. No evidence for PBBs-related effects on sperm counts, motility, or sperm morphology was found in a group of male Michigan workers exposed to PBBs by inhalation or dermal contact (Rosenman et al. 1979). No relationship was found between serum levels of PBBs and the frequency and duration of lactation in women exposed during the Michigan contamination episode (Thomas et al. 2001).

Although no alterations in fertility or litter size were observed in mink fed PBB-containing diets prior to breeding and during pregnancy (Aulerich and Ringer 1979; Ringer et al. 1981) or in the F_1 or F_2 generations of female F_0 rats fed PBB-containing diets during postimplantation gestation through weaning (McCormack et al. 1981), implantation was completely blocked in 40–67% of female rats exposed by gavage to PBBs between Gds 0 and 14 (Beaudoin 1979). Additionally, a lengthening of the menstrual cycle and prolonged implantation bleeding with decreased serum progesterone were observed in two of seven female monkeys fed a PBB-containing diet prior to and during pregnancy (Allen et al. 1979; Lambrecht et al. 1978). The dosage causing these reproductive effects in monkeys was the lowest tested in any intermediate-duration study of PBBs. In addition, alterations of male reproductive organs in rats (Gupta and Moore 1979) and in a monkey (Allen et al. 1978) have been observed after intermediate-duration exposure to lethal oral doses of PBBs. Histopathological alterations were not observed in male or female reproductive organs after intermediate- or chronic-duration, oral exposure of rats or mice to nonlethal doses of PBBs (NTP 1983, 1992). The animal data suggest that PBBs may cause adverse effects on reproductive organs and their function(s) and that reproductive organ functions during the early phases of pregnancy may be particularly sensitive to PBBs. Additional studies in animals exposed by oral and other routes, including multi-generation studies with pre-breeding exposure to assess effects on fertility in both males and females, might help to further identify the reproductive processes affected by PBBs and to determine the dose-response relationships. Studies elucidating the NOAEL region and relative susceptibility of sensitive species (e.g., monkeys) to reproductive and developmental effects would be particularly useful, as these data could enable derivation of an intermediate oral MRL.

Polybrominated Diphenyl Ethers. Information on the reproductive toxicity of PBDEs is limited to a single one-generation oral study of decaBDE in rats that found no exposure-related functional effects (Dow Chemical Co. 1975; Norris et al. 1975a, 1975b). Tests of octaBDE and/or pentaBDE, particularly second-generation studies designed to assess effects on fertility in both sexes, would better characterize the reproductive toxic potential of PBDEs and assure the adequacy of the intermediate oral MRL.

Developmental Toxicity.

Polybrominated Biphenyls. No studies were located regarding developmental effects in humans or animals after inhalation or dermal exposure to PBBs. Studies of fetal mortality rates in Michigan (Humble and Speizer 1984) and of physical and neuropsychological development in Michigan children exposed *in utero* or in early infancy during the peak of the 1973 contamination episode (Schwartz and Rae 1983; Seagull 1983; Weil et al. 1981) did not conclusively correlate the ingestion of PBBs with

developmental effects. Oral acute-, intermediate-, and chronic-duration studies of FireMaster FF-1 or FireMaster BP-6 in several species have reported fetotoxic and developmental effects, including embryolethality or increased mortality among nursing young (Allen et al. 1979; Beaudoin 1977, 1979; Groce and Kimbrough 1984; Lambrecht et al. 1978; Luster et al. 1980), fetal malformations (Beaudoin 1977; Corbett et al. 1975; Waritz et al. 1977), growth retardation in offspring (Allen et al. 1979; Aulerich and Ringer 1979; Corbett et al. 1975; Groce and Kimbrough 1984; Lambrecht et al. 1978; McCormack et al. 1981; Meserve et al. 1992; Ringer et al. 1981), liver effects in offspring (Moore et al. 1978; Werner and Sleight 1981), and performance deficits in tests of operant behavior in offspring (Henck and Rech 1986; Tilson 1992). A limited amount of data is available for octabromobiphenyl and decabromobiphenyl mixtures, which indicates that these PBBs are less developmentally toxic than FireMaster FF-1 or FireMaster BP-6 (Millischer et al. 1980; Waritz et al. 1977). Because FireMaster FF-1 caused developmental effects in monkeys at the lowest dosage tested in any study of PBBs, a chronic oral MRL could not be calculated; studies determining developmental NOAELs in sensitive species, therefore, would be particularly relevant. Additional studies regarding inhalation or dermal exposure to PBBs might help to determine whether or not the developmental toxicity of PBBs is route-specific. Studies on the mechanism(s) of action of PBBs in different animal species may provide a better understanding of the physiological and biochemical basis for the developmental toxicity of PBBs and a better basis for extrapolating from animal data in the evaluation of the hazard presented by PBBs to the development of human fetuses and children.

Polybrominated Diphenyl Ethers. Oral developmental toxicity studies have shown no evidence of teratogenicity of deca-, octa-, and pentaBDE in rats and rabbits, although fetotoxic effects, including skeletal ossification variations at maternally toxic doses, have occurred (Argus Research Laboratories 1985b; Breslin et al. 1989; Dow Chemical Co. 1975, 1985; Life Science Research Israel Ltd. 1987; Norris et al. 1975a, 1975b; WIL Research Laboratories 1986). Although effects of gestational exposure to PBDEs included minimally increased post-implantation loss in rats and increased skeletal variations in rabbits at octaBDE doses as low as 10 and 15 mg/kg/day (Argus Research Laboratories 1985b; Breslin et al. 1989; Life Science Research Israel Ltd. 1987), respectively, the available evidence appears to adequately show that developmental toxicity is not a critical effect of concern for PBDEs.

Immunotoxicity.

Polybrominated Biphenyls. Information regarding immunological effects of PBBs in humans is equivocal. Some groups of investigators reported altered lymphocyte transformation responses in

subjects accidentally exposed to PBBs through contaminated food (Bekesi et al. 1978, 1985; Roboz et al. 1985). Other investigators could not confirm this in the same populations (Landrigan et al. 1979; Silva et al. 1979). Carefully designed follow-up studies of these populations would provide valuable information regarding possible immunological effects of PBBs. Additional research on the binding of PBBs with different plasma fractions could be fruitful, since it appears that on a per cell basis in exposed subjects, there is . 100-fold excess of PBB in white cell fractions, compared to the erythrocyte fraction (Roboz et al. 1980, 1985). Acute oral data in rats and mice provided information regarding histopathology of the thymus, spleen, and lymph nodes (Fraker 1980; Fraker and Aust 1980; Gupta et al. 1981). Data from oral intermediate-duration studies in experimental animals suggest that the immune system may be one of the most sensitive targets for PBBs (Farber et al. 1978; Fraker 1980; Vos and van Genderen 1973, 1974). PBBs decreased the resistance of mice to infection by reducing antibody production (Loose et al. 1981), decreased the responsiveness of lymphocytes to mitogenic stimulation in rats and mice (Luster 1978, 1980) and pigs (Howard et al. 1980), altered thymus weight in rats (NTP 1983), and caused thymus atrophy in dogs (Farber et al. 1978), guinea pigs (Vos and van Genderen 1973), and cattle (Moorhead et al. 1977). No studies were located regarding the immunological effects of PBBs in animals after inhalation or dermal exposure. Due to the relatively low vapor pressure of PBBs, inhalation is not a predominant route of exposure. Additional oral studies using a battery of immunological tests would be useful to further define the immunological effects of PBBs.

Polybrominated Diphenyl Ethers. Information regarding the immunosuppressive potential of PBDE mixtures is essentially limited to evidence from acute-duration oral studies in animals exposed to relatively high doses of pentaBDE. The plaque-forming splenic cell antibody response to injected sheep red blood cells was significantly reduced in mice exposed to 72 mg/kg/day pentaBDE for 14 days (Fowles et al. 1994), and *in vitro* production of IgG immunoglobulin from pokeweed mitogen-stimulated splenocytes was reduced in mice exposed to 36 mg/kg/day pentaBDE for 14 days (Darnerud and Thuvander 1998). Other 14-day studies in mice found no changes in natural killer cell activity at #72 mg/kg/day (Fowles et al. 1994) or numbers of splenic and thymic lymphocyte subsets at #36 mg/kg/day (Darnerud and Thuvander 1998), although 18 mg/kg/day of the single congener 2,2',4,4'-tetraBDE caused significantly reduced numbers of total lymphocytes and CD4+, CD8+, and CD45R+ subtypes in the spleen (Darnerud and Thuvander 1998). Additional oral studies using a battery of immunological tests and a lower range of doses would serve to better characterize the immunotoxic potential of PBDEs.

Neurotoxicity.

Polybrominated Biphenyls. One study was located regarding neurological effects in humans after inhalation and/or dermal exposure to PBBs (Brown et al. 1981). No studies were located regarding neurological effects in animals after inhalation or dermal exposure to PBBs. Although neurological symptoms were reported with some frequency by certain residents of Michigan who were likely to have consumed PBB-contaminated food, several studies of Michigan residents (including workers in PBB manufacturing who presumably were exposed predominately by inhalation and dermal contact) found no statistically significant associations between levels of PBBs in serum or fat (from oral or dermal exposure to PBBs) and frequencies of subjectively reported neurological symptoms or performance on neuropsychological tests (Anderson et al. 1978c, 1979; Barr 1980; Brown and Nixon 1979; Brown et al. 1981; Landrigan et al. 1979; Stross et al. 1981; Valciukas et al. 1978, 1979). Studies of the neuropsychological development of children exposed *in utero* or in early infancy, likewise, were inconclusive in establishing an association with PBB exposure (Schwartz and Rae 1983; Seagull 1983; Weil et al. 1981). Subtle effects in neurobehavioral tests were found in rodents, including decreased motor activity (Geller et al. 1979; Tilson and Cabe 1979) and hind limb weakness (Cabe and Tilson 1978) after intermediate-duration, oral exposure and performance deficits in tests of learning behavior in the offspring of female mice and female rats exposed during gestation and lactation (Henck and Rech 1986; Henck et al. 1994; Tilson 1992). Histopathological alterations of brain or spinal nerve tissue revealed no abnormalities in rats or mice after intermediate- or chronic-duration oral exposure (NTP 1983, 1992). Periodic neurobehavioral testing of animals exposed to PBBs at multiple doses for chronic durations would be useful for determining if longer-term exposure leads to more severe neurological effects than those observed with intermediate-duration exposures.

Polybrominated Diphenyl Ethers. Very little information is available on the potential neurotoxic effects of PBDEs. Data are essentially limited to the results of three behavioral tests in animals showing some alterations in spontaneous locomotion behavior and learning and memory ability in mice that were tested at 2 months of age and as adults (4 months) following neonatal exposure (Pnds 3, 10, or 19) to single low oral doses of the congeners 2,2',4,4'-tetraBDE and 2,2',4,4',5-pentaBDE (Eriksson et al. 1998, 1999, 2001). Additional oral studies using a battery of neurobehavioral tests, particularly in developing animals, are needed to better characterize the potential for PBDEs to cause neurotoxic effects in humans.

Epidemiological and Human Dosimetry Studies.

Polybrominated Biphenyls. Epidemiology studies of people exposed by ingesting PBB-contaminated food as a result of the 1973 Michigan PBB contamination episode or who were exposed occupationally in the manufacture or distribution of PBBs have not provided conclusive evidence that detectable effects have occurred as a result of exposure to PBBs (Anderson et al. 1978c, 1979; Barr 1980; Henderson et al. 1995; Hoque et al. 1998; Humble and Speizer 1984; Landrigan et al. 1979; Thomas et al. 2001; Valciukas et al. 1978, 1979). Clinical examinations, including neuropsychological, liver function, and sperm count testing, of people who may have experienced the highest exposures did not conclusively identify particular effects or clinical signs associated with exposure (Brown and Nixon 1979; Brown et al. 1981; Rosenman et al. 1979; Schwartz and Rae 1983; Seagull 1983; Stross et al. 1981; Weil et al. 1981). No relationship was found between serum levels of PBBs and the frequency and duration of lactation in women exposed during the Michigan contamination episode (Thomas et al. 2001). A relationship between increasing serum levels of PBBs and increasing risk of breast cancer was indicated in case-control studies of women exposed during the Michigan episode (Henderson et al. 1995; Hoque et al. 1998), but the results are only suggestive due to factors such as small number of cases, insufficient information on known breast cancer risk factors, and confounding exposures to other organochlorine chemicals. The evidence for an association between breast cancer and PBBs is inconclusive and warrants further study. Continued monitoring of the Michigan cohort for prevalence of other types of cancer as the cohort ages are also of interest, because lifetime and short-term exposure to PBBs are known to cause cancer in animals, and the residence time of PBBs in the body is expected to be long. If human exposure to PBBs is found to be occurring at a hazardous waste site, the nearby population should be studied for both exposure and effect data.

Polybrominated Diphenyl Ethers. A limited amount of epidemiological information is available for PBDEs. Plasma levels of various organohalogen compounds, including the congener 2,2',4,4-tetraBDE, as well as serum hormone levels (free and total T_3 and T_4, TSH, free testosterone, follicle-stimulating hormone, lutenizing hormone, and prolactin), were analyzed in 110 men who consumed fatty fish from the Baltic Sea (Hagmar et al. 2001). There was a weak negative correlation between 2,2',4,4'-tetraBDE and plasma TSH after age adjustment, but 2,2',4,4'-tetraBDE could not explain more than 10% of the variance in TSH. No clear association was found between risk of non-Hodgkin's lymphoma and adipose tissue levels of 2,2'4,4'-tetraBDE in a case-control study of 77 Swedish men and women (Hardell et al. 1998; Lindstrom et al. 1998). 2,2'4,4'-TetraBDE was used as a marker for total PBDE exposure in both of these studies.

Animal data raise particular concern for effects of PBDEs on the thyroid as well as possible immunological, neurodevelopmental, and carcinogenic effects of exposure. Epidemiological investigations are needed to better characterize the potential for PBDEs to induce these and other kinds of effects. Considering the possibility that PBDEs can be transferred to the fetus across the placenta and that greater amounts might be transferred to nursing infants via breast milk, as well as evidence that perinatal exposure to PCBs and other similar chemicals may induce subtle neurological damage and immunological and thyroid effects in children, transgenerational studies would be particularly informative. Limitations that are likely to constrain the epidemiological investigations, such as unmeasured PBDE exposure concentrations and lack of controls for confounding co-exposures, should be addressed.

Biomarkers of Exposure and Effect.

Exposure.

Polybrominated Biphenyls. PBBs are stored primarily in adipose tissue and are present in serum and human milk of exposed populations. Several studies have shown that serum and adipose PBB levels are biomarkers of exposure (Brilliant et al. 1978; Humphrey and Hayner 1975; Lambert et al. 1990; Landrigan et al. 1979; Michels Blanck et al. 2000b; Rosen et al. 1995; Wolff et al. 1982). It has been proposed that measurement of PBB levels in adipose tissue may be a more reliable prediction of body burden than serum levels because of the high adipose/serum PBB partition ratio (Anderson 1985). However, once a stable correlation between adipose/serum levels has been characterized, serum levels are a better choice for surveillance and monitoring (Anderson 1985). Further studies on the predictive value of levels of PBB (particularly congeners) in serum and adipose tissue in individuals exposed to PBBs for acute, intermediate, and chronic durations would provide valuable information that could lead to early detection of PBB exposure.

A potential biomarker of exposure to PBBs is related to their effect on the thyroid gland. Effects in exposed workers included increased serum thyrotropin, low or borderline low serum T_4, and increased thyroid antimicrosomal antibody titers (Bahn et al. 1980), and effects in exposed rats included reduced levels of serum T_4 and T_3 (Akoso et al. 1982b; Allen-Rowlands et al. 1981; Byrne et al. 1987; Gupta and Moore 1979; Kasza et al. 1978a; Meserve et al. 1992; Norris et al. 1975a, 1975b; NTP 1983; Sepkovic and Byrne 1984; Sleight et al. 1978; Werner and Sleight 1981). Additional studies could better characterize thyroid effects of PBBs in humans and develop specific correlations between levels and duration of exposure and alterations in serum hormone levels.

Polybrominated Diphenyl Ethers. PBDEs also accumulate in adipose tissue, serum, and breast milk of the general population due to their lipophilic characteristics. Concentrations of PBDEs in breast milk are useful, non-invasive markers of maternal body burdens and of *in utero* and lactational exposures, but body burden assessments are limited by a lack of time-trend data for PBDEs in the milk of U.S. populations (Hooper and McDonald 2000). Breast milk monitoring programs are needed to provide time-trend data and to verify findings that PBDE levels have been exponentially increasing in breast milk during the past 25 years (Norén and Meironyté 1998, 2000). Studies on the predictive value of levels of PBDEs in serum and adipose tissue could provide useful information for detection and monitoring of exposure.

A potential biomarker of exposure to PBDEs relates to their effect on the thyroid gland. Thyroid changes in rats and mice include reduced serum T_4 levels with no changes in serum TSH (Darnerud and Sinjari 1996; Fowles et al. 1994; Hallgren and Darnerud 1998;WIL Research Laboratories 1984; Zhou et al. 2001, 2002). Additional studies could characterize thyroid effects of PBDEs in humans and develop specific correlations between levels and duration of exposure and alterations in serum levels of T_4.

Effect.

Polybrominated Biphenyls. There are no specific biomarkers of effects for PBBs. Numerous studies have attempted to correlate serum and adipose PBB levels with an array of symptoms and health complaints in PBB exposed subjects from the state of Michigan (Anderson et al. 1978a, 1978b, 1978c, 1979; Bekesi et al. 1978; Humphrey and Hayner 1975; Landrigan et al. 1979; Stross et al. 1979). Thus far, no significant correlation has been found. Continued follow-up studies on the Michigan cohort would provide information on effects that may have a long latency, such as cancer. Elevated levels of two cytochrome P-450I-dependent enzymes were observed among PBB exposed subjects, relative to controls (Lambert et al. 1990). The thyroid is a sensitive target for PBBs and characteristic changes include reduced serum levels of T_4 and other thyroid hormones (Akoso et al. 1982b; Allen-Rowlands et al. 1981; Byrne et al. 1987; Gupta and Moore 1979; Kasza et al. 1978a; Meserve et al. 1992; Norris et al. 1975a, 1975b; NTP 1983; Sepkovic and Byrne 1984; Sleight et al. 1978; Werner and Sleight 1981), indicating that they are potential biomarkers of effect. Levels of CYP enzymes and thyroid hormones, however, are not specific for PBB exposure. Further studies designed to identify specific biomarkers of effects of PBBs would facilitate medical surveillance leading to early detection of potentially adverse health effects and possible treatment. Congener-specific analysis may be useful for characterizing dioxin-like health effects.

Polybrominated Diphenyl Ethers. Biomarkers that could be used to characterize health effects caused specifically by PBDEs have not been identified. The thyroid is a critical target for PBDEs in animals, as discussed in Section 3.2.2.2, Endocrine Effects, and serum T_4 is a potential biomarker of effect for these chemicals in humans. Although this biomarker is not specific to PBDEs because other antithyroid agents can have similar effects, changes in T_4 could be considered to indicate potential impairment of health.

Absorption, Distribution, Metabolism, and Excretion.

Polybrominated Biphenyls. There are no quantitative data regarding absorption in humans via the inhalation route, but data from occupationally exposed individuals and individuals who ingested food contaminated with PBBs suggest that exposure by the oral or dermal route may lead to considerable accumulation of PBBs in tissues (Anderson et al. 1978c; Eyster et al. 1983; Landrigan et al. 1979). The animal data indicate that the main component of a commercial PBB mixture (2,2,'4,4',5,5'-hexa-bromobiphenyl) is efficiently absorbed by the oral route (Matthews et al. 1977; Tuey and Matthews 1980). Data regarding absorption after inhalation exposure was limited to a single study (Waritz et al. 1977). There are no data regarding absorption via the dermal route. No studies were located in which several doses of different PBB congeners were administered by the inhalation, oral, and dermal routes, and for various exposure periods. Such studies could provide information on the relationship between bromination patterns and absorption efficiency and rates of absorption by the different routes of exposure. In addition, studies with different PBB mixtures could help determine possible interaction effects among congeners that could affect absorption.

Distribution data are limited to qualitative information derived from cases of accidental ingestion of food contaminated with PBBs, cases of occupational exposure via dermal contact (Eyster et al. 1983; Landrigan et al. 1979) and autopsy reports (Miceli et al. 1985). These data suggest that PBBs distribute preferentially to tissues with high fat content regardless of the route of exposure. Data derived from oral administration of PBBs to animals indicate that PBBs are distributed first to liver and muscle and then to adipose tissue where they are stored (Domino et al. 1982; Lee et al. 1975; Matthews et al. 1977). Little information regarding distribution of PBBs could be drawn from the limited number of studies in animals administered PBBs by the inhalation or dermal routes. Additional well-conducted studies by these routes of exposure would provide useful information regarding possible route-dependent distribution patterns. Studies regarding distribution through the placenta after inhalation and dermal exposure were not available.

Data regarding biotransformation of PBBs in humans are limited to individuals who accidentally consumed food contaminated with PBBs or who were exposed to PBBs in the workplace (Wolff and Aubrey 1978; Wolff et al. 1979a). The use of human cell systems in culture might be considered a useful addition to whole animal studies for studying the metabolic fate of PBBs. There are studies regarding the metabolism of some PBB congeners after oral administration to rats (Sparling et al. 1980), rabbits (Kohli et al. 1978), and pigs (Kohli and Safe 1976). However, the PBBs mainly studied were monobromobiphenyls and dibromobiphenyls, which are only trace components of FireMaster mixtures. Therefore, studies on the *in vivo* metabolism of the main components of commercial PBB mixtures would provide valuable information regarding the metabolic disposition of highly brominated congeners. A limited amount of information is available on the metabolism of PBBs in farm animals (e.g., dairy cows, chickens). This is a data gap because people exposed to PBBs during the Michigan PBB contamination episode were predominately exposed by consuming products of farm animals. Although information regarding metabolism after inhalation or dermal exposure is lacking, there is no evidence to suggest that other pathways would operate after exposure by these routes.

Studies regarding urinary or fecal excretion of PBBs in humans were not located; however, elimination of PBBs through maternal milk is well documented (Brilliant et al. 1978; Eyster et al. 1983; Jacobson et al. 1984; Landrigan et al. 1979). Fecal excretion of unabsorbed PBBs appears to be the main route of elimination of highly brominated congeners after oral exposure (Matthews et al. 1977; Norris et al. 1975a; Rozman et al. 1982), whereas polar derivatives formed by lower brominated congeners appeared to be excreted mainly in the urine (Kohli and Safe 1976; Kohli et al. 1978; Sparling et al. 1980). Although data regarding excretion in animals after inhalation and dermal exposure were not located, there is no reason to believe that results from additional studies would reveal different patterns of excretion.

Polybrominated Diphenyl Ethers. A limited amount of data is available on the toxicokinetics of PBDEs. There are data gaps in a number of areas, particularly for octaBDE and pentaBDE mixtures and the tetra and hexa congeners that are most prevalent in the environment. Quantitative absorption studies could corroborate the conclusions on oral uptake in animals that are based on elimination and excretion data. Metabolism studies would help to characterize the enzymes involved as well as the transformation of some congeners to biologically active hydroxylated BDEs and the debromination of decaBDE to lower brominated BDEs.

Comparative Toxicokinetics.

Polybrominated Biphenyls. The data suggest that there are qualitative differences in the toxicokinetic disposition of PBBs among humans and among animal species (Wolff and Aubrey 1978; Wolff et al. 1979a). However, these differences appear to be highly dependent on the specific congener or mixture studied. In general, all species absorb PBBs, with varying efficiency, and accumulate PBBs in tissues rich in fat. Once absorbed, PBBs are distributed in a biphasic manner in all examined animal species (Domino et al. 1982; Ecobichon et al. 1983; Matthews et al. 1977). No studies were located that provide information regarding differences or similarities in metabolic disposition of PBBs between humans and animals. Limited data in humans indicate that fecal excretion of PBB residues occur (Eyster et al. 1983). Experimental data in animals suggest that the rate and extent of PBB elimination in the urine and feces are dependent on the degree and pattern of bromination (Kohli et al. 1978; Matthews et al. 1977; Sparling et al. 1980). Analysis of the excreta of humans exposed in the workplace and near hazardous waste sites would provide information regarding biotransformation and elimination kinetics in humans. In addition, similar target organs have been identified across animal species, but the database is not complete enough for identifying a most sensitive species. Although the toxicological data in humans are limited and inconclusive, adverse immune effects observed in humans (Bekesi et al. 1978) have also been observed in rats, mice, and pigs (Howard et al. 1980; Luster et al. 1978, 1980) suggesting that any of these species may represent a suitable animal model for humans. The only reported PBPK model for PBBs describes the distribution and body burden of the major component of FireMaster mixtures, 2,2',4,4',5,5'-hexa-bromobiphenyl, in the rat (Tuey and Matthews 1980). The serum mean half-life of 6.5 years predicted using this model is shorter than half-life values of approximately 12–29 years estimated using human sera data from the Michigan PBB cohort (Lambert et al. 1990; Michels Blanck et al. 2000b; Rosen et al. 1995). This indicates a need for an improved PBPK model for extrapolating animal data to humans and/or for studies designed to produce data for improving the performance of PBPK analyses.

Polybrominated Diphenyl Ethers. Insufficient data are available to determine whether qualitative differences in the toxicokinetic disposition of PBDEs exist between humans and animals and among animal species. Differences are likely to be dependent on the specific congener or mixture studied, and pharmacokinetic modeling studies could help to determine the validity of extrapolating data. Most of the available toxicokinetic studies of PBDEs have been performed in rats, and studies in other species could help to ascertain the most relevant animal model.

Methods for Reducing Toxic Effects.

Polybrominated Biphenyls. The mechanism by which PBBs enter the blood stream in humans is not known; consequently, there are no established methods for reducing absorption. Studies in experimental animals that could identify substances that prevent or delay absorption and that do not represent a toxic risk *per se* would be valuable. There are no established methods for reducing body burden in humans, but studies in animals and model simulations in humans indicate that reducing body fat markedly increases elimination of PBBs (Domino et al. 1982; Tuey and Matthews 1980). The effect of reduction of body fat (e.g., by dieting and exercising) in PBB-exposed humans has not been fully researched.

The mechanism of toxic action of PBBs is not completely understood and no methods exist to block the toxic response due to exposure to PBBs. A more complete characterization of the cytosolic Ah receptor protein, to which some PBB congeners are thought to bind, and understanding of physiological effects of receptor blockage would be useful for the possible identification of blockers of Ah receptor-mediated toxic effects. Further studies aimed at elucidating the nonreceptor-mediated mechanism of action of some PBBs would also be valuable.

Polybrominated Diphenyl Ethers. The mechanism by which PBDEs enter the blood stream is not known, there are no established methods for reducing body burden of PBDEs, and the mechanisms of toxic action of PBDEs are incompletely understood. Types of studies that could address these data gaps and possibly provide information on reducing toxic effects of PBDEs are discussed in the preceding subsection on PBBs.

Children's Susceptibility.

Polybrominated Biphenyls. Information on health effects of PBBs in children is available from several studies of the Michigan feed contamination episode. A 1976 study of Michigan children likely to have been exposed to PBBs found that the number of subjectively reported symptoms of ill health, including several symptoms of neurological effects, did not increase with increasing serum PBB levels, but rather decreased; general neurological examinations did not reveal a pattern of abnormality among the Michigan children (Barr 1980). Studies of fetal mortality rates in Michigan (Humble and Speizer 1984) and of physical and neuropsychological development in Michigan children exposed during the contamination episode (Schwartz and Rae 1983; Seagull 1983; Weil et al. 1981) did not conclusively correlate the ingestion of PBBs with effects. Neurobehavioral alterations have been observed in rats following

gestational and lactational exposure to PBBs (Henck and Rech 1986; Henck et al. 1994; Tilson 1992). Other effects in offspring of rats exposed to PBBs during gestation and lactation include decreased serum levels of thyroid hormone levels (Meserve et al. 1992; Werner and Sleight 1981). These effects in offspring are consistent with evidence that the thyroid gland is an unequivocal target of PBBs in adult animals. No information was located on possible immunological effects of PBBs in children, and data in adult humans are limited and largely inconclusive (Bekesi et al. 1978; Landrigan et al. 1979; Roboz et al. 1985; Silva et al. 1979; Stross et al. 1981), but exposure to PBBs caused altered immune responses in a variety of animal species, which suggests that children may also be affected. Continued assessment of children exposed to PBBs during the Michigan contamination episode, with particular emphasis on evaluation of cognitive abilities, thyroid function, and immune competence, would help to better assess the susceptibility of children to PBBs.

Polybrominated Diphenyl Ethers. No information was located on health effects of PBDEs in newborn or older children. Thyroid and neurobehavioral alterations have been observed in animals following pre- and/or postnatal exposure to commercial PBDE mixtures and single PBDE congeners, indicating that these are potential effects of concern in exposed children. Serum levels of thyroid T_4 and T_3 hormones were reduced in offspring of rats that were orally exposed to pentaBDE during gestation and lactation and in rats exposed as weanlings (Zhou et al. 2002). Alterations in spontaneous locomotion behavior and learning and memory ability were observed in mice that were tested at 2 months of age and as adults (4 months) following neonatal exposure to single low oral doses of the congeners 2,2',4,4'-tetraBDE and 2,2',4,4',5-pentaBDE (Eriksson et al. 1998, 1999, 2001). One of these studies found effects in mice treated at either 3 or 10 days of age, but not at 19 days of age, suggesting that there was a critical window for the induction of behavioral disturbances (Eriksson et al. 1999). Additional studies are needed to better characterize the potential susceptibility of children to the effects of PBDEs on the thyroid and neurodevelopment, particularly considering the possibility that these effects are related to the dependence of central nervous system development on thyroid hormones. No information is available regarding the immunosuppressive potential of PBDEs in children or young animals, indicating that studies of immune competence in developing animals would also help to more fully assess children's susceptibility to PBDEs.

Child health data needs relating to exposure are discussed in 6.8.1 Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

Ongoing studies that are relevant to health effects of PBBs and PBDEs, as identified in the Federal Research in Progress database (FEDRIP 2002) and the websites of various government agencies, are listed in Table 3-6.

Table 3-6. Ongoing Studies on the Health Effects of PBBs and PBDEs

Investigator	Affiliation	Research description	Sponsor
Hammock B	University of California, Davis, California	Support for testing hypotheses regarding the association of PBBs, PBDEs, and other known xenobiotic immunotoxicants and neurotoxicants with autism	NIEHS/EPA[1]
Huwe JK	Agricultural Research Service, Fargo, North Dakota	Development of effective remediation procedures for PBDEs and other persistent organic pollutants in animal tissues and their environment	USDA
Ludewig G	University of Kentucky, Lexington, Kentucky	A multispecies approach to analyze the toxic effects of PBDEs on organs and development	EPA
Palmer BD	University of Kentucky Medical Center, Lexington, Kentucky	Multidisciplinary/multispecies investigation of endpoints and mechanisms of action for PBDEs and PCDEs, including structure-activity relationships for endocrine disruption	NIEHS/EPA[1]
Robertson L	University of Kentucky Medical Center, Lexington, Kentucky	Laboratory synthesis of pure PBDE and PCDE congeners and their metabolites	NIEHS/EPA[1]
Sikka HC	State University of New York College at Buffalo, Buffalo, New York	Disposition and metabolism of PBDEs in fish	NOAA[2]
Trosko JE	Michigan State University, East Lansing, Michigan	Epigenic effects of PBBs and other environmental toxicants on cellular communication pathways	NIEHS/EPA[1]

[1]NIEHS/EPA Superfund Basic Research Program
[2]National Sea Grant College Program

EPA = U.S. Environmental Protection Agency; NIEHS = National Institute of Environmental Health Sciences; NOAA = National Oceanic and Atmospheric Administration; USDA = U.S. Department of Agriculture